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USAMRMC ltr, 19 Feb 2002

AD_____

Award Number: MIPR 5CKVFM5529

TITLE: Carcinogenesis of Depleted Uranium Fragments

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CONTRACTING ORGANIZATION: United States Department of Energy
Albuquerque, New Mexico 87185

REPORT DATE: June 2000

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	June 2000	Final (26 Jan 95 - 31 May 00)

4. TITLE AND SUBTITLE
Carcinogenesis of Depleted Uranium Fragments

5. FUNDING NUMBERS
MIPR 5CKVFM5529

6. AUTHOR(S)
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
United States Department of Energy
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
This report contains colored photos

12a. DISTRIBUTION / AVAILABILITY STATEMENT
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13. ABSTRACT ***(Maximum 200 Words)***

This study determined the carcinogenicity of depleted uranium [DU(Ti)] fragments in muscles of rats. Implants of DU(Ti) were compared to implants of tantalum (Ta), as a foreign-body control, and to injections of radioactive Thorotrast®, a colloid of thorium dioxide, as a radioactive control. The test metals, in four squares (2.5 × 2.5 × 1.5 mm or 5.0 × 5.0 × 1.5 mm) or pellets (2.0 × 1.0 mm diam.) were implanted in the muscles of male rats. Neither the type nor size of the implants affected survival. A fibrous capsule developed around the implanted metals, but not the injected Thorotrast®. Soft tissue sarcomas arose from some of the capsules and eroded the DU(Ti) implants. The erosion, noted radiographically, may be a diagnostically useful finding. The incidence of tumors was significantly increased in rats implanted with the largest DU(Ti) squares or injected with Thorotrast® when compared with Ta implants. These results in rats indicate that DU(Ti) fragments of sufficient size cause a local tissue reaction and can cause local sarcomas. The results do not indicate that DU(Ti) fragments are necessarily carcinogenic in humans. Individuals with large embedded DU(Ti) fragments, however, should be monitored carefully.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Depleted uranium	64
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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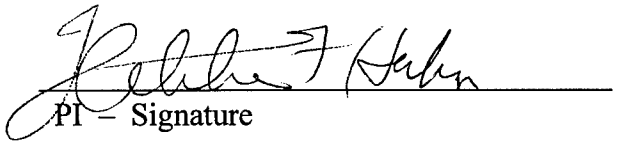

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April 20, 2001

Date

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INTRODUCTION

The purpose of this project was to determine the relative carcinogenicity of depleted uranium-0.75% Ti [DU(Ti); acronyms and symbols are defined in Appendix A] fragments embedded in soft tissues and the renal toxicity from chronic exposure to systemic uranium (U). These determinations were made from the study of rats exposed to embedded DU(Ti) fragments and comparison embedded materials. Two hypotheses were tested. First, we hypothesized that ***depleted U-0.75% Ti alloy fragments are more carcinogenic in muscle tissues than tantalum (Ta) metal fragments and less than a radioactive material, Thorotrast®***. The objective of testing this hypothesis was to determine the relative risk of radioactive fragments relative to non-radioactive fragments so that informed judgments can be made about the clinical management of veterans with DU(Ti) fragments embedded in their soft tissues.

The specific aim of this study was to determine experimentally the carcinogenicity of DU(Ti), a radioactive material Thorotrast®, and a non-radioactive inert metal, Ta. It is well known that rodents are more sensitive to foreign-body carcinogenesis than humans (Furst, 1981). Thus, the direct test of carcinogenicity in rats was rigorous and judged not to yield a false-negative result. On the other hand, a positive result cannot be extrapolated directly to the humans.

Second, we hypothesized that ***urinary concentrations of U are directly correlated with the renal concentrations of U and will reach a steady state after intramuscular implantation of DU(Ti) fragments.*** The objective of testing this hypothesis was to determine if the renal concentration of U reached a steady state and produced overt signs of toxicity. The specific aims of this study were to determine: 1) the time course to achieve a steady-state renal U concentration from an implanted DU source and 2) if toxicity was present at the steady-state concentration. In response to reviews before the project was funded, this portion of the project was restricted to data acquired from animals that were implanted with DU fragments and held for long periods. Accordingly, the scope of renal toxicity studies was limited.

BODY OF REPORT

I. Relative Carcinogenicity of DU Fragments

A. Pilot Carcinogenesis Study

The initial approach to determining the carcinogenic potential of DU was based on the model of foreign-body carcinogenesis in mice as used by Brand *et al.* (1975).

These investigators delineated the various etiologic factors and stages of foreign-body carcinogenesis by studying reactions in the subcutis of mice implanted with foreign bodies having large, smooth surfaces. The extensive and detailed work of these investigators laid a potential framework for testing the carcinogenicity of DU fragments.

It was necessary, however, to clarify a number of critical variables before using this model to study the carcinogenic potential of DU or DU(Ti). A pilot study addressed three of these critical variables by determining: 1) the *in vivo* solubility of DU during the first 60 days after its implantation in rats and mice, 2) the changes in the surface characteristics of the DU foil after implantation, and 3) histological responses of rats and mice to the implanted DU during this time. The experimental design is shown in Table 1.

Table 1
Experimental Design for the Study of Dissolution and Excretion of Uranium and Early Biological Effects of Subcutaneously Implanted DU, DU(Ti), or Ta Metal Foils in Male Rats and Mice

	Foil Type and Number of Animals Sacrificed at 30 Days			Foil Type and Number of Animals Sacrificed at 60 Days[a]			
Rodent	DU	DU(Ti)	Ta	DU	DU(Ti)	Ta	Total
F344 Rats	5	5	4	5	5	4	28
CBA/J Mice	5	5	4	5	5	4	28
Total	10	10	8	10	10	8	56

[a]Twenty-four-hour urine samples were collected from three rats and three mice on days –2, –1, 1, 2, 3, 4, 7, 14, 21, 28, 35, 42, 49, 56, and 60 before and after DU and DU(Ti) foils were implanted and on days –2, 7, 14, 28, and 35 before and after Ta foils were implanted, respectively.

Two types of foils containing DU were used. One contained only DU, the other in which DU was alloyed with 0.75% Ti [DU(Ti)]. The foils measured approximately 20 mm × 15 mm × 1.5 mm. The DU foils weighed 8.4 ± 0.3 g, and the DU(Ti) foils weighed 7.4 ± 0.2 g. Ta foils of similar size were used as control implants. The Ta foils weighed 5.6 ± 0.1 g. The composition of the DU(Ti) foils was the same as that used in a study at the Armed Forces Radiobiology Research Institute (AFFRI) on the dissolution of DU(Ti) pellets in rats (Castro *et al.*, 1996) and has been described in detail (Daxon, 1995).

The daily U excretion data for individual rats is summarized in Figure 1 (all figures are in Appendix B). The urinary excretion of U appeared to increase throughout the

study in rats with implanted DU foils. In contrast, the excretion of U by rats with implanted DU(Ti) increased rapidly until about 15 to 20 days, after which the daily excretion was relatively constant. The concentrations of U in the kidneys of rats with DU and DU(Ti) foil implants followed a similar pattern, i.e., at 30 and 60 days greater concentrations of U were in the kidneys of rats with the DU(Ti) implants than in the rats with the DU foil implants (Figure 2). The translocation of U to the kidney and skeleton also indicated that the DU(Ti) foils were more soluble that the DU foils.

Similar urinary excretion patterns were seen in mice, except that the rate of excretion of U in the mice implanted with DU (Ti) was not constant after 15 to 20 days as in the rats, but continued to increase (Figure 3). The dissolution of U in the mice with the implanted DU(Ti) resulted in the accumulation of toxic levels of U in the kidneys (Figure 4) resulting in the death of all but one mouse within 30 days, compared with the death of only one rat with implanted DU. Histopathological examinations of the kidneys showed a chronic tubular necrosis, which was severe enough to cause death before the 60-day sacrifice. The severity of the lesions was generally correlated with the concentration of U in the kidney.

Thirty days after implantation in the subcutis, the physical appearances of both the DU and the DU(Ti) foils were markedly altered (Figure 5). The surfaces were roughened and friable, with small black particles and flakes coming off the foils. The flaked particles blackened the lining of the connective tissue capsule surrounding the foils. The appearance was accentuated at 60 days. The DU and DU(Ti) foils were surrounded by a moderately thick connective tissue capsule with infiltration of chronic inflammatory cells (Figure 6). Black particles were embedded in the capsule.

The results of the pilot study indicated that DU and DU(Ti) foils reacted similarly, but not identically, in the subcutis of mice and rats. U concentrations in the kidneys increased throughout the 60-day observation period. The DU(Ti) foils dissolved more rapidly than DU. In addition, both types of DU foils broke down in the subcutis, becoming roughened and causing a moderate inflammatory cell infiltration in the surrounding tissues. DU(Ti) foils caused more inflammation and more renal damage. Both of these effects most likely relate to the greater solubility of DU(Ti). The results indicated that DU alloyed with the 0.75% Ti should be used in the carcinogenicity testing.

These results made evident that the subcutaneous foreign-body carcinogenesis model system described by Brand *et al.* (1975) could not be applied to a study of

the carcinogenesis of DU(0.75% Ti) fragments. Key elements in the Brand foreign-body carcinogenesis model are a smooth surface on the foreign body and a relative lack of inflammatory response. Therefore, results of the pilot study indicated that another approach must be taken to assess the carcinogenic potential of DU compounds.

B. Carcinogenesis Bioassay Study

A long-term carcinogenesis bioassay study with intramuscularly implanted DU(Ti) pellets and fragments was designed to determine the carcinogenic potential of DU. The study design was patterned after the National Toxicology Program Statement of Work and the EPA Guidelines 40 CFR 798: 3320 - "Combined Toxicity and Oncogenicity Testing."

A recent report by the International Agency for Research on Cancer notes that available studies are inadequate to permit reliable and accurate estimates of the long-term effects of DU in humans (McGregor *et al.*, 2000). However, laboratory studies have indicated that DU fragments may be carcinogenic. For example, rats implanted in the muscles with 20 DU pellets (2.0 × 1.0 mm diameter) excreted uranium in the urine for at least 18 months (Pellmar *et al.*, 1999). Urine from these rats had enhanced mutagenic activity in *Salmonella typhimurium* strain TA98 and the Ames II™ mixed strains (TA7001-7006) (Miller *et al.*, 1998a). The mutagenicity increased in a dose- and time-dependant manner with a strong positive correlation with urinary uranium concentration. In addition, DU-uranyl chloride transformed immortalized human osteoblastic cells to the tumorigenic phenotype (Miller *et al.*, 1998b). DU-uranyl chloride treatment resulted in a 9.6-fold increase in neoplastic transformation frequency compared with untreated control cells. In comparison, nickel sulfate, a known human carcinogen, resulted in a 7.1-fold increase in transformation frequency. These findings of mutagenesis and neoplastic transformation suggest that DU may be carcinogenic.

To help clarify the potential carcinogenicity of DU fragments in the soft tissues, a long-term bioassay study with implanted materials was conducted in rats. The study included both radioactivity (Thorotrast® injection) and foreign-body (tantalum metal implant) control groups. The results indicate that DU fragments cause localized tumors in rats at a higher incidence than the Ta fragments but at a lower incidence than the Thorotrast®.

1. Materials and Methods

The carcinogenicity of intramuscularly implanted DU (alloyed with 0.75% Ti) was determined using a long-term bioassay study. The sizes and shapes of the

DU used were similar to the range of sizes and shapes of DU fragments embedded in soldiers wounded in the Gulf War (Daxon, 1995). Cylindrical DU pellets were obtained from Manufacturing Sciences Corporation, Oak Ridge, TN. Two sizes of DU fragments were cut from DU foil obtained from the same source. The physical characteristics of the pellets and fragments used in the study are shown in Table 2.

Table 2
Physical Characteristics of Pellets and Fragments Implanted in Rats

Implant	Number	Volume[a] (mm^3)	Mass[a] (mg)	Surface Area[a] (mm^2)	E alpha[b] (nCi)
DU(Ti) Pellet	1 pellet	1.6	30	7.9	0.16
2.0 × 1.0 mm diameter					
"	4 pellets	6.4	119	31.4	0.64
DU(Ti) Fragment	1 fragment	9.4	175	27.5	0.54
2.5 × 2.5 mm					
"	4 fragments	37.5	698	110	2.2
DU(Ti) Fragment	1 fragment	37.5	698	80	1.6
5.0 × 5.0 mm					
"	4 fragments	150	2790	320	6.4
Ta Fragment	1 fragment	27.5	456	72	–
5.0 × 5.0 mm					
"	4 fragments	110	1824	228	–
Thorotrast® Injection	1 site	0.057	–	–	3.1
"	2 sites	0.114	–	–	66.2

[a]Equations:

	Volume	Surface Area	Mass
Pellet	$V = \frac{\pi D^2 L}{4}$	$S = \pi DL + \frac{\pi D^2}{2}$	$M = V \times \rho$
Fragment	$V = L^2 T$	$S = 2L^2 + 4LT$	

where: D = diameter, L = length, T = thickness, ρ = density of DU(Ti) = 18.6 mg/mm^3.

[b]E alpha = effective alpha-particle activity emanating from the surface of the DU(Ti) or Thorotrast® in nCi. See text.

The mass of DU(Ti) fragments used varied by a factor of 20, and the surface area varied by a factor of 10. Brand *et al.* (1976) have noted that foreign bodies, smooth, solid pieces of metals, plastics, or glass, may induce tumors when implanted in the subcutis of rats. Size (surface area) was one important determinant of this type of carcinogenicity. Based on the results of Alexander and Horning (1959) relating carcinogenicity to foreign-body size, the maximum size of 5.0 × 5.0 mm was chosen to minimize the occurrence of nonspecific tumors. Fragments (5.0 × 5.0 mm) of Ta (Goodfellow Corp., Berwyn, PA) were used as a foreign-body control. In addition, the fragments were implanted in muscle, not subcutis, to further minimize nonspecific tumors. The intramuscular route is commonly used to evaluate the carcinogenicity of metals and metal compounds (Furst, 1981).

Thorotrast®, a 25% colloidal thorium dioxide (^{232}Th) radiographic contrast media, was used as a positive control for radioactive materials. The distribution, retention, and carcinogenic effects of Thorotrast® have been summarized (van Kaick *et al.*, 1995; Machinami *et al.*, 1999). Thorotrast® is reported to cause granulomas if injected perivascularly, which occurred inadvertently in a few human patients. A small fraction of these lesions developed into sarcomas (Dahlgren, 1967; Liebermann *et al.*, 1995). The Thorotrast® used in this study was produced by Hyden Chemical Corp, NY, and kindly supplied by Jim Humphreys, AEA Technologies, Harwell, England.

Two sources of alpha irradiation of muscle soft tissue were used in this study, one from the metallic uranium implants, and the second from injected colloidal Thorotrast®. During the planning stages, the radiation dose rates to tissue within range of the alpha particles emitted by either the uranium- or thorium-series isotopes were estimated as follows.

Alpha spectrometric characterization of the DU used in this study indicated that the only alpha-emitting isotopes present in significant abundance were ^{238}U and ^{234}U; the ^{234}U/^{238}U activity ratio was 0.14. Because the range of the uranium alpha particles in the dense uranium metal is very small (a few μm) compared to the dimensions of the implant (e.g., 5.0 × 5.0 × 1.5 mm for the largest implant), it was convenient to measure the alpha-particle flux being emitted from the surfaces of the DU(Ti) metal implants. Using a ZnS alpha-particle detector, the emission rate of alpha particles from the U was measured to be 4240 disintegrations per minute (dis minute^{-1}) per cm^2 of implant surface. Assuming an average alpha energy per emitted particle of 82 MeV (an acknowledged overestimate because of the infinitely thick source

geometry), a radiation dose rate averaged over a 50-μm tissue thickness surrounding the implant (adequate to capture all alpha particle energy emitted by both uranium- and thorium-series isotopes) was calculated to be 82 rad day^{-1}. Recognizing that the alpha radiation dose rate varies significantly within the 50-μm thickness of tissue, it was nonetheless useful to calculate this tissue-averaged dose for comparison with that produced by Thorotrast®.

Calculating an alpha radiation dose from injected Thorotrast® was more complicated than for the uranium metal implants because of the complex thorium decay series of isotopes. When the progeny of ^{232}Th are in equilibrium with the parent, six alpha particles are emitted per decay of ^{232}Th (^{228}Th, ^{224}Ra, ^{220}Rn, ^{216}Po, and either ^{212}Bi [36%] or ^{212}Po [64%]). Because the Thorotrast® used in this study was produced during the 1950s and remained in sealed vials until its use in this study, it was reasonable to assume that ^{232}Th and its progeny were essentially in equilibrium prior to use. However, subsequent to intramuscular injection, it was no longer appropriate to assume that the radioactive progeny of ^{232}Th remain at the injection site, because 1) a fraction of the non-thorium progeny exists in the solution phase rather than in the Thorotrast® particles, and the particles were transported from the injection site as soluble species; and 2) newly created progeny had finite probabilities of being ejected from the Thorotrast® particles by recoil mechanisms, and thus also become available for translocation as soluble atoms. Parr *et al.* (1969) measured the state of equilibrium of the progeny of ^{232}Th in various tissues from Thorotrast®-injected humans, dogs, and rats and found that the steady-state ratios of isotopes were not related to species for important tissues such as liver, spleen, and red bone marrow, the major deposition/retention sites for intravenously injected Thorotrast®. The alpha radiation doses in this study were as follows: $^{228}Ac/^{228}Ra = 0.97$, $^{228}Th/^{228}Ra = 0.89$, $^{224}Ra/^{228}Th = 0.53$, $^{212}Pb/^{224}Ra = 0.48$, and $^{212}Bi/^{212}Pb = 0.70$. The only ratio from Parr *et al.* that was not used was the $^{228}Ra/^{232}Th$ of 0.27. Because the Thorotrast® used in this study was maintained for about 40 years in a sealed vial, a $^{228}Ra/^{232}Th$ ratio of 0.9 was assumed. As such, the number of alpha particles emitted per disintegration of ^{232}Th was 3.18, and the total amount of alpha-particle energy available for deposition at the wound site was 16.95 MeV per disintegration of ^{232}Th. To calculate the alpha-radiation dose rate soon after injection, it was assumed that an initial volume of 0.025 cm^3 containing 1510 dis $minute^{-1}$ ^{232}Th was injected. However, because the original injection volume was >90% aqueous vehicle which would be absorbed soon after injection, the geometry would become a point or line source of Thorotrast®. The actual geometry of the deposited Thorotrast® particles at the injection site is not known and

likely varied among sites. Lacking experimental data, the self-absorption factor of 0.26 published by Brooks *et al.* (1986) for Thorotrast® deposited in liver tissue was used. This factor is probably low and hence the dose rate overestimated, but probably less than a factor of two. Using these assumptions, a net energy deposition rate of 767 MeV minute^{-1} was deposited in a 50-μm thick shell of tissue immediately in contact with the Thorotrast®. This deposition rate corresponds to 104 rad day^{-1}, which was about 1.2 times the dose rate from the DU(Ti) implants. Within the uncertainties in the calculations, the soft tissue alpha dose rates are essentially the same.

Six groups of 50 male Wistar rats were treated with one of the following: DU(Ti) pellets (2.0 mm length × 1.0 mm diameter), DU(Ti) fragments (2.5 × 2.5 × 1.5 mm) or (5.0 × 5.0 × 1.5 mm), Thorotrast® injection (0.050 mL), Ta fragments (5.0 × 5.0 × 1.1 mm), or sham surgery (Table 3). Three-hundred, 12-week-old, male Wistar rats were purchased (Charles River Laboratories, Wilmington, MA) and maintained in the Lovelace Respiratory Research Institute (LRRI) animal facility. The Wistar strain was chosen because of its relatively large size, making surgical implants easier, and its relatively low incidence of chronic nephropathy (Short and Goldstein, 1992).

Table 3
Experimental Design: Carcinogenesis Bioassay Study of DU(Ti) Pellets and Fragments Intramuscularly Implanted in Male Wistar Rats

Type of Implant	Size (mm)	Number of Implants	Total Number of Rats
DU(Ti) pellets	2.0 × 1.0 diameter	4	50
DU(Ti) fragments	2.5 × 2.5 × 1.5	4	50
DU(Ti) fragments	5.0 × 5.0 × 1.5	4	50
Ta fragments	5.0 × 5.0 × 1.1	4	50
Thorotrast® injection	0.050 mL	2	50
Sham implant surgery	NA	0	50
Total number of rats	–	–	300

The rats were housed, two per cage, in filter-topped polycarbonate cages on hardwood chip bedding. Animal rooms were maintained at 20 to 22°C with a relative humidity of 40 to 60%. Teklad Certified Rodent Diet (Harlan Teklad, Madison, WI) and water

were available *ad libitum*. Cages and bedding were changed twice per week. The rats were individually identified with tail tattoos and randomly placed into groups of 50 rats.

Before implantation surgery, the DU(Ti) pellets, DU(Ti) fragments, and Ta fragments were weighed, cleaned to remove the oxide formation, and sterilized. Cleaning and sterilization comprised immersion in an industrial detergent, rinsing in absolute ethyl alcohol, immersion in a 50% nitric acid solution for 3 minutes, and rinsing with sterile water. All pieces were stored in absolute ethyl alcohol to inhibit oxidation. The DU(Ti) and Ta fragments were implanted in the *biceps femoris* muscle of each hind leg, two fragments per leg. Sterile procedures were used to incise the skin and muscle for the implant site. The incisions were closed with two absorbable sutures in the muscle and three surgical wound clips in the skin. Because Thorotrast® was a colloid suspension, it was injected into the *biceps femoris* muscle (two injections of 0.05 mL, one in each hind leg). Radiographs were taken immediately after implantation or injection to verify the placement of the test materials.

After implantation of the fragments, the rats were held for observation for the length of the experiment, 2 years. A Biological Sciences Peer Group review of the study held on February 16, 1999, recommended that the observations be extended for the normal life span. The U.S. Army Medical Research and Material Command subsequently provided funds to allow lifetime observations.

All rats were weighed prior to the implantation of the test materials, then weekly for 6 weeks and monthly thereafter until death. At each weighing, clinical observations were made, including palpation of the implant sites. Representative animals were selected for radiographs at 3–4 weeks and 1 year after implantation. Rats were observed twice daily, and those moribund or in distress were euthanized with pentobarbital according to LRRI standard protocols.

At death or euthanasia, complete necropsies were performed on all animals. Radiographs were taken to locate the implanted or injected test materials and observe the skeletal structures. The implant sites and the draining lymph nodes were examined for lesions, and the implants were removed. The abdominal, thoracic, and cranial cavities were opened and all organ systems examined. Representative tissue sections were taken, fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 micra, and stained with hematoxylin and eosin. Histological examination was routinely performed on the implant sites, the draining lymph nodes, implant site neoplasms, gross lesions that were potential neoplasms,

and the kidneys, urinary bladder, prostate, seminal vesicles, testicles, epididymis, spleen, quadriceps femoris muscle, liver, and lungs. The soft tissue tumors were classified histologically based on the criteria of the Society of Toxicologic Pathologists (Greaves *et al.*, 1992). Other lesions were generally classified using previously published criteria (Boorman *et al.*, 1990).

The quadratic weights curves were fit for each rat using PROC NLIN (SAS, Version 8.0, SAS Institute, Cary, NC). Three rats were not used due to incomplete data, one each in the B, H, and Q groups. Parameter estimates were output and compared using analysis of variance (ANOVA). The survival distribution function was estimated for each group using the nonparametric Kaplan-Meier method. The log rank and the Wilcoxon tests were used to evaluate homogeneity across all groups. If homogeneity was rejected ($p < 0.05$) by either test, pair-wise comparisons were made (SAS Proc, Version 8). The differences in the tumor incidences among the various exposure groups were compared using Fischer's exact test (Sigma Stat for Windows Version 1.0, SPSS Science, Chicago, IL).

2. Results

The body weights for the various exposure groups are shown in Figure 7. The increase in weight in early life with a decrease in later life is a pattern often observed with male rats (Rao *et al.*, 1990). The 5.0 × 5.0 mm DU(Ti) fragment group had shape parameter estimates for the weight curve that significantly differed from the sham control group. On average, this group reached its maximum weight sooner than the sham group and at a slower rate of increase. This finding indicated that these larger fragments did cause some systemic toxicity. The exact nature of this toxicity could not be determined. It may have related to a subtle renal toxicity (see p. 26 on renal toxicity).

The survival of the various exposure groups did not differ significantly (Figure 8). The median survival time for the six groups ranged from 576 to 620 days after implantation. The similarity of the survival of the six groups eliminated a competing risk when determining tumor incidence.

The radiographic appearance of the implanted DU(Ti) fragments changed markedly during the first year. At the time of implantation, the fragments were smooth squares with regular, sharp, well-defined edges (Figure 9A). At 21 days after implantation, small, dense blebs extended from the edges of the fragments, making them appear larger than at the time of implantation. The jagged appearance disrupted the sharp profile of the fragments

(Figure 9B). At 1 year after implantation, the radiographic profiles of the fragments were rounded, with no corners and a fine, jagged edge (Figure 9C). At the time of death, many of the profiles were enlarged up to 1.5 times in linear dimensions. The smaller DU fragments had similar, but less severe changes. These radiographic changes were related to surface corrosion of the DU(Ti) *in vivo* and capsule formation. In contrast, the profiles of the Ta fragments were smooth at all times with sharp, well-defined edges and did not increase in size with time (Figures 10A and B). Initially, the Thorotrast® injections gave a spherical radiographic outline (Figure 11A). At 4 weeks, the profile was irregular and diffuse, with no distinct boundary (Figure 11B). The profiles had a similar appearance at 1.5 years after injection (Figure 11C).

The implants of DU(Ti) or Ta were encapsulated with connective tissue at the time of death. Most of the fragments were located in the muscles where they were originally implanted, but some had migrated to the loose connective tissues between the muscles of the leg. The injected Thorotrast® did not induce capsule formation, but was localized in and around the muscles causing a tan discoloration. Before histologic sectioning of the capsules, the implants were removed. The Ta fragments slipped easily from the capsules. The DU(Ti) fragments, however, adhered to the capsules and were difficult to remove from the tissues. The surfaces of these fragments were friable. A black, gritty layer of granular material was left on the inner surface of the capsule.

The capsules around the DU(Ti) implants were characterized histologically by fibrosis, inflammation, degeneration, and mineralization (Figure 12A). Around the large DU(Ti) implants, the capsules were up to 0.5 mm thick and composed of dense fibrous tissue. More typically, the capsules were 0.1 to 0.2 mm thick. Shards of black material were embedded in the fibrous tissue capsules around the medium and large fragments. Smaller particles, in a range of sizes (<4 μm), were found in the capsule wall around all sizes of DU(Ti) implants. Chronic inflammatory cells and particle-laden macrophages were frequently scattered throughout the capsule wall of the DU(Ti) implants. Occasionally foreign-body giant cells were found. However, the amount of chronic inflammation in and around the capsules was generally similar with DU(Ti) implants of all sizes. Degeneration of the fibrous tissue in the capsule wall was frequent at the interface with the implant. With this reaction, the tissue on the inner surface of the capsule was devitalized and necrotic. The lumen of the capsules contained necrotic and proteinaceous debris, scattered acute inflammatory cells, and varying amounts of black shards or particles. The particles were contained in macrophages or free in the lumen as amorphous

clumps of various sizes. Mineralization of necrotic debris or devitalized fibrous tissue on the inner wall of the capsules was common.

The capsules around the Ta implants were characterized by fibrosis with little inflammation, and no degeneration or mineralization (Figure 12B). The capsule walls were less than 0.1 mm thick with a smooth inner surface. No shards or particles were present.

The Thorotrast® lesion was an accumulation of macrophages between muscle fibers and adjacent to muscles (Figure 12C). The macrophages were filled with a tan, coarsely granular material. These macrophages were not associated with inflammation or fibrosis.

Soft tissue tumors of various types were associated with many of the implants (Table 4).

Table 4
Rats with Implant-Associated Soft Tissue Tumors

Animal No.	DPI[a] (days)	Death Mode	Tumor Type	Metastasis	Relation to Death
DU(Ti) 5.0 × 5.0 mm Implant					
F 081	715	M Sac[b]	MFH[c] in capsule wall (microscopic)	None	INC[d]
F 089	767	M Sac	MFH	None	COD[e]
L 090	611	M Sac	MFH	None	COD
L 092	493	M Sac	Osteosarcoma	None	COD
L 095	535	M Sac	MFH	None	COD
L 099	582	M Sac	Fibrosarcoma	Lumbar LN[f]	COD
R 093	696	M Sac	MFH	None	COD
R 094	635	M Sac	MFH	None	COD
R 100	572	M Sac	Osteosarcoma	None	COD
DU(Ti) 2.5 × 2.5 mm Implant					
E 068	515	Died	Benign fibrous histiocytoma in capsule	None	INC
E 071	601	Died	Right leg - MFH	None	COD
			Left leg - Fibroma w/osseous metaplasia	None	INC
K 080	855	M Sac	Fibrosarcoma in capsule (microscopic)	None	INC
Ta 5.0 × 5.0 mm Implant					
H 034	592	M Sac	MFH	None	COD
N 028	987	M Sac	MFH in capsule (microscopic)	None	INC

Table 4
Rats with Implant-Associated Soft Tissue Tumors (concluded)

Animal No.	DPI[a] (days)	Death Mode	Tumor Type	Metastasis	Relation to Death
Thorotrast® Injection					
C 034	792	M Sac	Fibrosarcoma	None	COD
C 036	670	M Sac	MFH	None	COD
C 037	562	M Sac	MFH	None	COD
C 038	547	M Sac	Fibrosarcoma	None	INC
C 040	728	M Sac	MFH	None	COD
C 042	698	M Sac	MFH	None	COD
C 045	660	M Sac	MFH	None	COD
C 046	593	M Sac	MFH	None	COD
C 047	799	M Sac	MFH	None	COD
C 048	666	M Sac	Fibrosarcoma	None	COD
I 038	645	M Sac	Osteosarcoma - Soft tissue on X-ray	Lung	COD
I 041	541	M Sac	Fibrosarcoma	None	COD
I 042	679	M Sac	MFH	None	COD
I 043	666	M Sac	MFH	None	COD
I 044	518	M Sac	MFH	None	COD
I 045	586	M Sac	Fibrosarcoma	None	COD
I 048	705	Died	MFH	None	COD
I 050	621	M Sac	Fibrosarcoma	None	COD
I 051	799	M Sac	MFH	None	COD
O 040	714	M Sac	Fibrosarcoma in muscle (microscopic)	None	INC
O 042	712	M Sac	Fibrosarcoma	None	COD
O 044	645	M Sac	MFH in muscle (microscopic)	None	INC
O 045	769	M Sac	Myoblastoma in muscle (microscopic)	None	INC
O 046	622	M Sac	Fibrosarcoma	None	COD
O 051	607	M Sac	Fibrosarcoma (microscopic)	None	INC

[a]Days post-implantation.
[b]Moribund sacrifice.
[c]Malignant fibrous histiocytoma.
[d]Incidental.
[e]Cause of death or sacrifice.
[f]Lymph node.

All tumors were in the soft tissues of the hind legs, directly associated with the implanted DU(Ti) or Ta fragments or the injected Thorotrast®. Grossly, the neoplasms appeared to grow primarily by expansion, rather than invasion. On the cut section, black fragments of the implanted DU(Ti) could be found scattered through the tumor tissue (Figure 13).

Histologically, three tumors were localized to the wall of the fibrous capsules surrounding the implants. In other tumors, black shards, particles of implanted fragments, or Thorotrast®-filled macrophages could be seen scattered through most of the tumor tissues. These histologic findings lend further credence to the association of the implants with the tumors.

The most commonly found tumor types were malignant fibrous histiocytomas and fibrosarcomas. The three osteosarcomas noted were not associated with the skeleton. Although there was a higher number of fibrosarcomas in the Thorotrast®-treated rats and a broader range of tumors in the DU-treated rats, a specific tumor type could not be attributed to a specific treatment. All of the tumors, however, were most likely derived from pluripotent mesenchymal stem cells (Brooks, 1986).

Biologically, these tumors were moderately aggressive (Table 5). Many were rapidly growing, expanding in size from barely palpable to 3 or 4 cm in 2 weeks. However, none invaded bone, and only one ulcerated the skin. One metastasized to an iliac lymph node and another metastasized widely. Twenty-nine of the 40 were large enough to result in euthanasia. The other 11 were discovered at necropsy or in tissue sections of the capsules surrounding the implants.

Table 5
Biological Characteristics of Implant-Associated Soft Tissue Tumors

	Total Number of Tumors	Relationship to Death		Number with Metastasis	Median Survival Time (days)	Range (days)
		COD[a]	INC[b]			
Benign Tumors						
Fibrous histiocytoma, benign	1	0	1	0	515	–
Fibroma	1	0	1	0	601	–
Granular cell myoblastoma	1	0	1	0	769	–
Malignant Tumors						
Fibrous histiocytoma, malignant	22	18	4	0	668	518–987
Fibrosarcoma	12	8	4	1	614	515–853
Osteosarcoma	3	3	0	1	572	493–645

[a]Cause of death or euthanasia.
[b]Incidental.

The incidence of the tumors was increased in the rats with the largest DU(Ti) implants when compared with the sham or foreign-body (Ta) controls (Figure 14). The difference was significant ($p \leq 0.028$) using a Fischer's exact test. The control animals injected with Thorotrast® had a significant increase in number of tumors compared with the DU(Ti)-implanted rats ($p < 0.0014$). The DU(Ti)-treated rats had a fragment size-related response. The response could not be explained by physical surface area alone because the tumor incidence with the Ta implants of similar size was much lower. There was, however, a correlation with the initial surface alpha radioactivity. This initial activity was calculated from the physical characteristics of the DU(Ti) fragments and the Thorotrast® colloid. The injected colloid was considered a sphere for calculation purposes. Radiographs showed that the physical shape of the fragments and the colloid changed within 4 weeks after implantation. Thus, the surface alpha radioactivity changed with time as the shape of the implants changed.

Comparison of the radiographs of the DU-associated lesions with the histologic appearance of the lesions showed a correlation. After being implanted for a year or more, all DU(Ti) fragments were enlarged and rounded in radiographic profile (Figure 15A). These features generally correlated with a dense, connective tissue capsule. However, disruption of the smooth-edge profile and focal loss of density in the DU(Ti) fragment were associated with proliferative lesions or small tumors in the capsules (Figure 15B). Disintegration and breakup of the DU fragment were apparent on radiographs when frank tumors were present. On the histologic sections, black shards of DU(Ti) could be seen throughout the tumor tissue (Figure 15C). These radiographic changes associated with the DU(Ti) fragments may be important indicators of accompanying proliferative lesions and may have prognostic value in clinical evaluations.

The tumors in tissues not associated with the implant sites are noted in Table 6. There was no significant increase related to exposure in any of the tissues routinely sampled or in tissues sampled when gross lesions were noted. The numbers of renal tumors was increased in those rats with implants of DU(Ti). The incidence was not statistically significant, however, even when all groups implanted with DU(Ti) fragments combined were compared with all three control groups (sham, Ta, Thorotrast) combined ($p = >0.06$ with Fischer's exact test).

Primary renal tumors were found in five rats and were classified as tubular cell adenoma – 1, tubular cell adenocarcinoma – 3, and sarcoma, not otherwise specified – 1. The biologic characteristics of these tumors are noted in Table 7. Two renal adenocarcinomas were found in the 5.0 × 5.0 mm group, which had a mean concentration of 10.5 μg/g kidney (SD ± 6.92). A renal adenoma and an adenocarcinoma were found in the 2.5 × 2.5 mm group, which had a mean concentration of 8.95 μg/g kidney (SD ± 5.38). A renal sarcoma was found in the 2.0 × 1.0 mm diam. group, which had a mean concentration of 2.17 μg/g kidney (SD ± 3.03). The U concentration in the kidney of rats with renal tumors ranged from 0.173 to 4.49 μg/kidney.

Table 6
Number of Benign and Malignant Tumors Not Associated with Implant Site

	Sham	Ta Fragment	Thoro-trast®	DU(Ti) 2.0 × 1.0 mm	DU(Ti) 2.5 × 2.5 mm	DU(Ti) 5.0 × 5.0 mm
Number of Rats Examined	50	50	50	50	50	49
Muscle	0/0[a]	0/1	0/0	0/0	0/1	0/0
Kidney	0/0	0/0	0/0	0/1	1/1	0/2
Urinary bladder	0/0	0/2	0/0	0/0	0/1	0/0
Testes	5/0	7/0	3/0	3/0	5/0	5/0
Prostate	0/0	0/0	0/0	0/0	0/0	0/0
Epididymis	0/0	0/0	0/0	0/0	0/0	0/0
Seminal vesicle	0/0	0/0	0/0	0/0	0/0	0/0
Liver	4/0	4/2	1/1	1/1	4/1	1/2
Spleen	0/6	0/4	0/1	0/2	0/7	0/4
Lung	0/0	0/0	0/0	0/0	0/0	1/0
Number of Rats Examined – Varied; tissues sampled if gross lesions present						
Bone	0/0	0/0	0/0	0/2	0/0	0/0
Adrenal	1/2	2/1	0/3	0/1	0/2	5/1
Thyroid	1/0	2/2	0/1	3/0	2/0	0/0
Pituitary	6/5	6/2	18/1	6/0	9/3	4/1
Mammary gland	3/0	3/1	3/0	2/0	4/0	1/0
Pancreas	1/0	2/1	0/4	2/0	4/0	1/2
Skin	7/1	5/2	5/3	5/3	4/1	7/1

[a]Number of benign tumors/number of malignant tumors.

Table 7
Biological Characteristics of Renal Tumors

	Total Number of Tumors	Relationship to Death		Number with Metastasis	Median Survival Time (days)	Range (days)
		COD[a]	INC[b]			
Benign Tumors						
Tubular adenoma	1	0	1	0	577	–
Malignant Tumors						
Tubular adenocarcinoma	3	2	1	1	751	642–803
Sarcoma NOS[c]	1	1	0	0	644	–

[a]Cause of death or euthanasia.
[b]Incidental.
[c]Not other specified.

3. Discussion

These findings clearly indicated that DU fragments of sufficient size were carcinogenic in the muscles of rats. The incidence of soft tissue neoplasms was significantly greater in the rats with DU(Ti) implants than in rats with the foreign-body control (Ta) implants. The neoplasms induced were associated directly with the implants; no increase in tumors was noted elsewhere in the body.

The validity of injecting or implanting materials and compounds in the subcutis or muscles of rodents to test for carcinogenicity in humans has been questioned (Furst, 1981). The concern is that tissue reactions seen in rodents are seen with many materials, may be nonspecific, and may not occur in humans. Several compounds or materials injected or implanted in the subcutis of rats caused localized cancer, but were later determined not to cause cancer in humans. Examples are certain food colorings (Grasso and Golberg, 1966), iron dextran (Baker *et al.*, 1961), and silicone implants with breast cancer (McGregor *et al.*, 2000). In addition, seemingly innocuous materials placed in the subcutis of rodents have caused localized cancers, materials such as aluminum foil, glass sheets, and methyl cellulose filters of a certain size (Brand *et al.*, 1976). This phenomenon has been termed foreign-body carcinogenesis.

Foreign-body carcinogenesis has been induced experimentally in the soft tissues of rats by a number of sheet-like implanted materials indicating that rats are sensitive to this type of carcinogenesis (Greaves *et al.*, 1985; Autian *et al.*, 1975; O'Gara *et al.*, 1967; Oppenheimer *et al.*, 1956). The physical nature of the sheets was more important than the chemical nature in determining carcinogenesis (Brand *et al.*, 1976). The size of the sheet (greater than about 5.0 × 5.0 mm), physical smoothness (a non-corroded surface), and continuity (no holes in the sheet) were three key physical parameters. A sheet of sufficient size to be carcinogenic could be rendered non-carcinogenic by cutting it into smaller pieces, perforating it with numerous holes, or roughening the surface. The degree of inflammation also played a role. The greater the inflammatory response or the longer it persisted in the tissues, the less likely the sheet-like material would induce soft tissue tumors.

Some have used the term "solid-state carcinogenesis" to describe the type of foreign-body carcinogenesis that requires sheet-like materials and specific physical parameters to be operative (Bishoff and Bryson, 1964). The term separates this peculiar form of carcinogenesis seen in rodents from other types of foreign-body carcinogenesis caused by dyes

and food additives (Grasso and Golberg, 1966). It is also apparent that solid-state carcinogenesis is not the process involved in the DU(Ti) fragment-related soft tissue sarcomas.

Tests using implanted materials in rats, however, can be reasonable predictors of the carcinogenicity of materials in humans. Numerous metal powders and metal implants have been tested by injection or embedding in the muscles of rats (Table 8). A range of localized tissue responses was noted related to chemical composition or size. These findings indicate that not all metals injected or implanted in the muscles of rats induce localized tumors and that the element is important for metal powders, and the size (or surface area) is important for the metal squares, discs, or rods.

Table 8
Carcinogenesis Studies of Metals Implanted or Injected in the Muscles of Rats

Material	Form	Dimension (mm)	Mass or Surface Area	Tumor Incidence[a]	Reference
DU(Ti) – metal	Implant – pellet	2.0 × 1.0	7.9 sq mm	0/50	This study
DU(Ti) – metal	Implant – square	2.5 × 2.5 × 1.5	28 sq mm	3/50	This study
DU(Ti) – metal	Implant – square	5.0 × 5.0 × 1.5	80 sq mm	9/49	This study
Tantalum	Implant – square	5.0 × 5.0 × 1.1	72 sq mm	2/50	This study
Stainless steel	Implant – rod	8.0 × 1.6	44 sq mm	0/34	Gaechter *et al.*, 1977
Vitallium – cast or wrought	Implant – rod	8.0 × 1.6	44 sq mm	0/49	Gaechter *et al.*, 1977
Titanium – unalloyed	Implant – rod	8.0 × 1.6	44 sq mm	0/24	Gaechter *et al.*, 1977
Titanium – alloy	Implant – rod	8.0 × 1.6	44 sq mm	0/22	Gaechter *et al.*, 1977
Stainless steel	Implant – disc	4.0 × 1.5	25 sq mm	0/40	Stinson, 1964
Stainless steel	Implant – disc	12.0 × 1.5	75 sq mm	2/37	Stinson, 1964
Stainless steel	Implant – disc	18.0 × 1.5	113 sq mm	5/42	Stinson, 1964
CoCrMo – metal alloy	Powder		28 mg	0/142	Meachim *et al.*, 1982
CoCrMo – metal alloy	Powder		28 mg	27/72	Swanson *et al.*, 1973
NiFe – metal	Powder		14 mg	0/20	Sunderman, 1984
Cr – metal	Powder		4 mg	0/20	Sunderman, 1984
Lead metal	Powder		95 mg total	1/37	Furst and Harding-Barlow, 1979
Ti – metal	Powder		23 ♀ or 39 ♂ mg	2/50	Furst, 1971
Co – metal	Powder		28 mg	17/30	Heath, 1956
Ni – metal	Powder		25 mg total	38/50	Furst and Harding-Barlow, 1979
Ni – metal	Powder		28 mg	10/10	Heath and Daniel, 1964

[a]At site of implant or injection.

For the metal powders, the incidence of localized tumors ranged from 0 to 100%, depending on the element. The highest incidence was with nickel, a known human carcinogen. The lowest incidences were with metals with low carcinogenicity, titanium,

lead, and chromium. For implanted materials, there appeared to be a relationship to the size, or surface area. For example, all of the metals with surface areas of 44 sq mm or less did not induce localized tumors, with the exception of DU(Ti) 2.5 × 2.5 mm fragments with 28 sq mm used in the study reported here. The stainless steel implant with 75 sq mm had a tumor incidence of 5%, essentially the same as the tumor incidence for Ta fragments with 72 sq mm. On the other hand, the DU(Ti) 5.0 × 5.0 mm fragments with nearly the same surface area of 80 sq mm had a tumor incidence of 18%.

The finding of DU(Ti) fragment carcinogenicity in rats does not indicate, however, that DU(Ti) fragments are necessarily carcinogenic in humans. Artificial implants and accidental foreign-body material are associated with occasional soft tissue sarcomas in humans, although precise figures are not available (Jennings *et al.*, 1988). It has also been argued that the rate must be low because of the small number of implant-associated soft tissue sarcomas reported in the literature in spite of the frequent use of various implants for medical purposes (Brand, 1994). The sole epidemiological study of artificial implants and soft tissue sarcomas used a case-control approach with a population of 217 Vietnam veterans with soft tissue sarcomas (Morgan *et al.*, 1995). Respondents noted if they had an implant of an artificial joint, pin, plate, staple, screw, or any other metal or plastic implant. None had an artificial joint. In that study, no association was found between soft tissue sarcomas and the implants.

Uranium compounds have not been demonstrated to cause cancers in humans, even as a result of high occupational exposures (Harley, 2000). Over 30,000 persons occupationally exposed in U.S. Department of Energy contractor laboratories have been followed in epidemiological studies with no health effects reported. These exposures were primarily by inhalation or ingestion, not by the embedding of metal fragments. Thus, the exposure of the Gulf War veterans who were wounded with DU(Ti) fragments is unique.

The mechanism by which DU(Ti) fragments induced localized cancers in rats may be important in determining the carcinogenicity of DU(Ti) fragments in humans. The Ta fragments used here fit two criteria for foreign-body carcinogenesis associated with solid-state materials: smoothness and continuity of the sheet. Little inflammation was induced by the Ta fragments, and the fibrous capsule was thinner than the capsule around the DU(Ti) fragments. The size (5.0 × 5.0 mm) was smaller than that reported necessary for solid-state carcinogenesis in rats (Alexander *et al.*, 1959). However, the tumor incidence was

essentially the same as that for stainless steel disc implants of similar surface area (Table 8). The soft tissue tumors related to the Ta fragments in this study were most likely induced by the foreign-body mechanism described in rodents implanted with solid-state materials (Brand *et al.*, 1976).

The Thorotrast® injections induced a high incidence of soft tissue sarcomas, indicating that rats are sensitive to carcinogenesis from radioactive materials implanted in the muscles. The ^{232}Th in Thorotrast® is radioactive. The radioactive decay chain from ^{232}Th results in about six alpha particles per disintegration with an average total energy of about 26 MeV. The calculated radiation dose to the local tissue around the injection site in the rats is about 380 gray per year based on the injection initially being a sphere with the volume of 0.50 mm^3 and soon becoming a point or linear source. This calculated radiation dose is only an estimate, and is probably low compared to the real value. This annual dose is much greater than the estimate of ~1 gray per year for the human patients who had Thorotrast® inadvertently injected into the paravascular soft tissues (Liebermann *et al.*, 1995). These individuals developed intense sclerotic lesions in the soft tissues, and one (out of 245) developed a soft tissue sarcoma. In the Wistar rats, essentially no inflammatory reaction, fibrotic reaction, or proliferative lesion was seen in response to the injected Thorotrast®. However, the earliest deaths, where tissues could be examined, were at about 300 days after injection so that an early, transient inflammatory reaction may have been overlooked.

The DU(Ti) fragments did not appear to cause soft tissue sarcomas by the foreign-body mechanism of solid-state materials so well described in rats (Brand *et al.*, 1976; Bishoff *et al.*, 1964). The surface of the DU(Ti) fragments, although initially smooth, quickly became corroded and roughened. In addition, the histologic reaction to DU(Ti) fragments showed much more inflammation and fibrosis than did the lesions associated with Ta fragments. The Ta fragments used in this study and the stainless steel discs used in previous studies (Stinson, 1964) initiated tumors by the foreign-body mechanism and had a much lower incidence of sarcomas. These differences between the reactions and tumor incidence of DU(Ti) and Ta fragments or stainless steel discs indicate that something more than solid-state carcinogenesis is occurring with DU(Ti)-fragment carcinogenesis.

A radiation mechanism may have played a role in the carcinogenicity of the DU(Ti) fragments. One indication of this was the increased incidence of soft tissue sarcomas, which was correlated most closely with the increased surface alpha

radioactivity of the implanted materials than with the surface area (Figure 14). On the other hand, Thorotrast®, which initiated tumors by a radiation mechanism, elicited essentially no reaction in the tissues, a distinct difference from the DU(Ti) fragments.

The mechanism by which DU(Ti) fragments induced soft tissue sarcomas is uncertain. One striking feature of the reaction is the corrosion of the DU(Ti) in the tissues and the intensity of the inflammatory and fibrotic response to the fragments. The response is similar to the local tissue reactions produced by food coloring and other soluble compounds repeatedly injected subcutaneously in rats (Grasso and Golberg, 1966). Those compounds that produced tissue destruction with subsequent dense collagen formation invariably induced soft tissue sarcomas. Physicochemical properties closely related to tissue destruction were marked surface activity, lipid solubility, and protein-binding ability (Gangolli *et al.*, 1967). These properties are not directly related to chemical carcinogenesis but to a continual cellular damage and repair mechanism. A similar process may occur with DU(Ti)-associated soft tissue sarcomas.

Understanding the mechanism by which DU(Ti) fragments induce tumors in rats is important in determining the likelihood of a similar mechanism occurring in humans. Further research will be required to determine the importance of various mechanisms: foreign body, radiation, chemical, or continued cell damage and repair.

The lack of finding a significantly increased incidence of tumors other that at the site of the implants indicates that U is not an effective systemic carcinogen. The tumor types and incidence were similar to those previously described for Wistar rats (Bomhard, 1992; Tucker, 1997). The incidence of primary renal tumors was 4% in each of two DU(Ti) fragment groups (5.0 × 5.0 mm and 2.5 × 2.5 mm). This incidence was high related to that previously reported in Wistar rats (Bomhard, 1992). The increase, however, was not statistically significant. In addition, the types of tumors found were similar to those occurring spontaneously in Wistar rats.

The implications of these findings on the medical management and risk assessment for wounded veterans are unclear. The finding of DU(Ti) implant-associated soft tissue sarcomas in rats indicates a carcinogenic potential for DU(Ti) fragments. However, based on the findings in the controls in this study and the reports in the literature (McGregor *et al.*, 2000; Brand *et al.*, 1976), rats are much more sensitive to foreign-body and radiation carcinogenesis than are humans. The findings from these studies cannot be directly extrapolated

to humans. More information is needed before risk estimates for humans with embedded DU(Ti) fragments can be determined.

Prudence, however, requires caution in the medical management of wounded individuals. It may be prudent to use radiography to monitor embedded fragments in wounded individuals. Changes in the radiographic profiles of the fragments that show focal loss or breakdown and changes associated with proliferative lesions in the rats may indicate removal of specific fragments.

II. Renal Toxicity from Chronic Exposure to Systemic Uranium

A. *In Vitro* Dissolution Study

The biokinetics and dosimetry of embedded DU fragments depend to a significant extent on the physicochemical properties of the DU *in vivo*. The more insoluble the DU fragments, the greater the dose to the implant site and the lesser to the kidney. Conversely, as solubility increases, the dose to the kidney increases, as does the potential for toxicity. It is, therefore, important to measure the dissolution rate of DU *in vivo* and *in vitro*. The former provide data needed for biokinetic/metabolic modeling, whereas the latter can provide insight into the mechanisms of dissolution of U present as metal fragments. These results of an *in vitro* dissolution study were designed to supplement the *in vivo* dissolution results obtained in rats after intramuscular implantation of DU fragments.

1. Materials and Methods

Two types of DU metal fragments were used: 1) DU fragments 1 cm × 1 cm in size, 0.15 cm thick; and 2) DU containing 0.75% titanium DU(Ti) of the same size. Both materials were obtained from Manufacturing Sciences Corp. (Oak Ridge, TN) as foils and were cut into the requisite sizes at LRRI using a standard mechanical shear. Ta fragments run in parallel *in vitro* dissolution systems were used as control fragments.

The *in vitro* measurements were done by placing one of the DU or DU(Ti) fragments into a LRRI static dissolution cell (Kanapilly and Goh, 1973), which consisted of a "sandwich" of two 47-mm hydrophilic polysulphone membrane filters (Tuffryn-HT, 0.2 μm pore size, Pall Gelman Laboratory, Ann Arbor, MI) and a screw-fastened Teflon ring that holds the filters securely around the test material, the DU fragments. The static cell was immersed in a volume of 50 mL of solvent and maintained at room temperature without stirring. At selected times (4 hours; 1, 4, 8, 11, 18, 22, 25, and 28 days), the solvent was exchanged with an equal

volume of fresh solvent. At the termination of the dissolution study (28 days), the dissolution cell containing the undissolved U was removed from the solvent, disassembled, photographs taken, and the various parts of the dissolution system submitted for U analysis (see below). The two solvents used in this study were synthetic serum ultrafiltrate (SUF; Eidson and Griffith, 1984) and distilled, deionized water adjusted to pH 5.0 ± 0.1 with dilute HCl. The composition of SUF is noted in Table 9. The pH of the SUF solutions was maintained at 7.4 ± 0.1 by maintaining an atmosphere of 5% CO_2 in air in the head space above each liquid sample. The pH was checked routinely throughout the course of the experiment. The pH 5 solvent was monitored in a similar manner, except that no CO_2 was added to the atmosphere.

Table 9
Composition of Synthetic Serum Ultrafiltrate (SUF)

Solute	Molar Concentration
NaCl	0.116
NH_4Cl	0.010
$NaHCO_3$	0.027
Glycine	0.0050
$Na_3Citrate$	0.0002
$CaCl_2$	0.0002
L-Cysteine	0.001
H_2SO_4	0.0005
Na_2HPO_4	0.0012
DTPA[a]	0.0002
ABDAC[b]	50 ppm

[a]Diethylenetriaminepentaacetic acid, not present in blood serum was used to minimize binding of solubilized actinides to container and apparatus surfaces.
[b]Alkylbenzyldimethylammonium chloride was added as an antibacterial, antifungal agent.

All samples were analyzed for U content using a kinetic phosphorescence analyzer (KPA-11, Chemchek Instruments, Inc.). The lower limit of detection is 50 ng L^{-1}. Aliquots of the solvent samples were taken "as is" and measured directly. The filter and filter holder samples were ashed at 550°C and dissolved in 1 M HNO_3. The remaining

insoluble U fragment and associated particles were dissolved in concentrated nitric acid, then diluted to 1 M HNO_3 for aliquoting and U measurement.

During the course of the *in vitro* dissolution study, it was noted within 4 days that the exterior surfaces of the filters became progressively discolored. For the SUF samples, the discoloration consisted mainly of gray areas, which appeared to be associated with the location of the U fragments [both DU and DU(Ti)]. For the pH 5 samples, the discoloration pattern was more complex, with gray areas occurring together with yellow and orange areas. These discolorations were most intense in the region of the U fragment, but extended beyond the fragment margin, and in some cases, discolored the filter holder with a yellow deposit as well. This latter deposit was firmly affixed to the plastic. In the same time, very small black grains began to appear within the solvent. The origin of this material is not known. It appears unlikely that visible U particles could have permeated the membrane filters where the pore size was 0.2 μm. Nor is it known whether the black particles were indeed U. However, the presence of this particulate material, if U, would tend to bias the dissolution results upward, as this material was analyzed along with the solvent. Additional information could be obtained by repeating the *in vitro* study and analyzing the particles with appropriate microchemical techniques, e.g., electron energy loss spectrometry or secondary ion mass spectrometry.

2. Results

When the dissolution study was terminated and the filter holders disassembled, a significant quantity of very fine particulate material was found within the filter "sandwich," along with the remaining U fragment (Figures 16–19). The surface of the fragment itself was severely corroded, and the edges of the fragment were smoothed down. Both the fragment and the particles were generally black, with a brownish cast also noted in the pH 5 samples. No yellow or orange material was observed within the sample volume contained by the filters. There appeared to be a greater quantity of particles in the pH 5 samples than in the SUF samples. The exact meaning of the observed color changes is not known. However, it is surmised that the various changes are indicative of a multi-step fragmentation-dissolution process whereby U metal is transformed into soluble uranyl ions (UO_2++).

The *in vitro* dissolution data were best fitted to single-component exponential functions. The results of the fitting are summarized in Table 10 and the data and

curves illustrated in Figures 20–23. Each curve contains the data from the respective duplicate samples. Two trends are identifiable: 1) both forms of DU were more soluble in pH 5 solvent, and 2) DU was somewhat more soluble in either solvent than DU(Ti).

Table 10
Single-Component Exponential Fit Parameters for *In Vitro* Dissolution Samples

Material	Solvent	Intercept (%)	Rate Constant ($days^{-1}$)	Half Life (days)
DU	SUF	99.8	0.00425	163
DU(Ti)	SUF	99.9	0.0025	277
DU	pH 5	101.5	0.0113	61.0
DU(Ti)	pH 5	101	0.0085	81.5

In the first case, the DU and DU(Ti) samples dissolved, respectively, two and three times more rapidly in pH 5 solvent. Although comparative *in vivo* bioassay data are not available to date, it is expected that the dissolution rates obtained with the SUF solvent will better approximate the *in vivo* rates. This is based on the fact that SUF was originally formulated to mimic the chemical composition of extracellular fluids such as serum. The pH 5 solvent on the other hand was designed to model the intraphagolysosomal pH of alveolar macrophages, and thus would be most appropriate for inhalation exposures. Because the DU fragments were embedded in muscle, it is expected that the chemical milieu will more closely resemble extracellular fluid. Thus, the half-times of 163 days for DU and 277 days for DU(Ti) are believed more relevant.

It is interesting to note that the addition of 0.75% Ti to the DU appears to have decreased its solubility in these aqueous test environments. However, the differences in the two solvents, although statistically significant, are less than a factor of two, and do not change one's impression of the intrinsic solubility of the DU metal. In all cases, the solubilities would be described as moderate in the parlance of respiratory tract dosimetry models, i.e., type M absorption class (ICRP, 1994) or class W solubility (ICRP, 1979). There is no comparable dosimetry model for wounds, such as an implant site.

It is also important to note that the *in vivo* excretion results from the pilot study (see following section) do not agree with the above *in vitro* data. *In vivo*, more U from DU(Ti) was excreted in the urine, compared to DU. In addition, the levels of U in the

kidneys of the DU(Ti) rats and mice were also higher. Both indicate that DU(Ti) was more soluble *in vivo* than *in vitro*. The reasons for these differences are presently not understood.

B. *In Vivo* Distribution Study

The second hypothesis of the project was that urinary concentrations of U are directly correlated with the renal concentrations of U and will reach a steady state after intramuscular implantation of DU(Ti) fragments. The objective of testing this hypothesis was to determine if the renal concentration of U reaches a steady state and produces overt signs of toxicity. The specific aims of this study were to determine: 1) the time course to achieve a steady-state renal U concentration from an implanted DU(Ti) source and 2) if toxicity is present at the steady-state concentration. In response to reviews prior to funding of the project, this portion of the project was restricted to data acquired from animals that were implanted with DU(Ti) fragments and held for long periods. Accordingly, the scope of the renal toxicity study was limited.

The objective of the study was to determine daily urinary excretion of U at intervals over a 1.5 year period, and U concentration in the kidney and carcass.

1. Materials and Methods

Rats from the carcinogenesis bioassay were used in part of the study. Refer to Carcinogenesis Bioassay Study (section I.B., p. 8) for details on the implantation and general care of the animals. Six of the rats with 5.0 × 5.0 × 1.5 mm DU(Ti) fragments were randomly chosen for urine collections. The same six rats were sampled over an 18-month period. Twenty-four hour urine samples were collected from the rats as they were housed in stainless steel metabolism cages. The rats were conditioned in these cages for 2 weeks before implantation and for 48 hours before each sampling period. After each sampling period, the cages were washed to remove any adherent U. The U detected in the washes was added to the amounts found in the urine for that sampling period.

In addition, 36 rats were implanted with 2.5 × 2.5 × 1.5 mm DU(Ti) fragments and sacrificed at various intervals to determine the distribution of U in the kidney and carcass at relatively short periods after implantation. Groups of four rats were sacrificed 10 days, 1, 2, 4, 9, 12, and 18 months after implantation.

Clinical chemistry and hematology determinations were also performed at the time of sacrifice. Blood samples were obtained from each rat by cardiac

puncture at terminal sacrifice. Blood samples for clinical chemistry (1.5 mL) were collected into microtube serum separator tubes for centrifugation and separation of cell and serum fractions. The clinical chemistry analyses were performed with a Monarch 2000 (Instrumentation Laboratories, Lexington, MA). The specific analytes were albumin, alkaline phosphatase, blood urea nitrogen, calcium, creatinine, creatine kinase, inorganic phosphorous, and total protein.

Blood samples for hematology (1 mL) were collected into microtubes containing ethylenediaminetetraacetic acid. Hematology analyses were performed with a Baker 9110 Plus hematology analyzer (BioChem ImmunoSystems, Allentown, PA). Parameters analyzed were red blood cell count, hemoglobin, hematocrit, platelet count, and white blood cell count. Differential cell counts were performed manually.

Tissue samples from rats on the carcinogenicity and serial sacrifice studies were obtained at necropsy for determination of U content. At necropsy the right kidney and carcass from all rats were taken for U analysis. The carcass was prepared by depelting the remainder of the animal after the encapsulated DU(Ti) fragments and all the organs were removed for examination. Thus, the carcass was composed of the skeleton and attached muscles. Previous studies have shown that most U in the carcass of rodents is located in the skeleton, not the muscle or other soft tissues (Tannenbaum and Silverstone, 1951).

Urine samples and washes from metabolism cages, kidney, and carcasses were analyzed for U using the Kinetic Phosphorescence Analyzer, KPA 11 (Chemchek Instruments, Inc., Richland, WA). LRRI experience with the KPA 11 indicates that the practical limit of detection for U was 0.05 μg/L. The samples were prepared for analysis by dry ashing at 550°C, wet digestion in concentrated nitric acid, treating with 3 M hydrofluoric acid and 0.2 M H_3BO_3, and dilution in 1 N nitric acid.

2. Results

The daily excretion of U in the urine at various times after implant is noted in Figure 24. There was a steep increase in the U content of the urine over the first 30 days after implantation. In five of the six rats, this was over 200 μg/day. After 50 days, the U excretion slowly decreased from an average of about 50 to 25 μg U per day. The rats that died before the 550 days collection period showed a lower excretion in the several months before death. However, none of these rats died with obvious renal toxicity, nor did their kidneys have high U concentrations relative to others in the exposure group.

The daily fraction of the implant that was excreted in the urine is shown in Figure 25. The excreted fraction was about 2×10^{-5} and slowly decreased to about 6×10^{-6}. The average excreted fraction over the life span of all six rats was $1.4 \times 10^{-5} \pm 5.5 \times 10^{-6}$. The renal concentration of U at death related to the daily fraction of the implant is noted in Figure 26. This graph illustrates poor correlation of these two parameters.

The concentration of U in the kidney at various times after DU(Ti) fragment implant is shown in Figure 27. The results are from rats that were euthanized or died on the carcinogenesis bioassay; thus, the earliest values are from about 300 days after implantation. From this time until 800 days, the concentration of U in the kidney did not increase appreciably. In rats in the pellet group, the mean renal concentration was 2.72 ± 3.03 μg/g, in the 2.5×2.5 mm group it was 8.95 ± 5.38 μg/g, and in the 5.0×5.0 mm group it was 10.48 ± 6.92 μg/g. The renal concentrations in the two groups with the larger fragments were not significantly different, in spite of the differences in mass (~3X) and surface area (~2X).

Histopathology of the kidneys from the rats on the carcinogenesis bioassay study is summarized in Table 11. Chronic nephropathy, a common renal lesion in aged rats of many strains (Gray, 1986), was ubiquitous in the rats that lived out their normal life span. The lesion is characterized by progressive involvement of glomeruli and tubules, with thickening of basement membranes, focal tubular regeneration, mononuclear inflammatory cell infiltrates, glomerular hyalinization and sclerosis, tubular hyaline casts, interstitial fibrosis, mineralization, and cyst formation. Chronic nephropathy obscured any subtle effects from U in the kidney of essentially all the bioassay rats. Pyelitis, an inflammation of the pelvis of the kidney, was usually associated with cystitis and was marked in about half the cases. It was more prevalent in the Thorotrast® group, but no association could be made with DU(Ti) implants.

In the serial-sacrifice study, the renal concentration of U was measured in rats sacrificed from 10 days to 18 months after implantation of 2.5×2.5 mm DU(Ti) fragments (Figure 28). The renal U concentration increased from 1.4 μg/g 10 days after implantation to 8.4 μg/g at 1 month. The concentration increased gradually to 10.9 μg/g at 6 months, then decreased to 4.2 μg/g at 12 months and 6.7 μg/g at 18 months.

Table 11
Renal Lesion Incidence and Severity Grades
Carcinogenesis Bioassay Study

		Sham	Ta	Thorotrast®	DU(Ti) Pellet	DU(Ti) 2.5 × 2.5	DU(Ti) 5.0 × 5.0
	Number in Group	50	50	50	50	50	49
Kidneys	Number Examined	50	50	50	48	50	49
Nephropathy	NAD[a]	2	0	1	0	0	0
	Minimal	4	4	8	4	7	3
	Mild	6	11	9	10	7	7
	Moderate	5	7	10	6	8	7
	Marked	33	28	22	28	28	32
	Total Incidence	48	50	49	48	50	49
Cyst	NAD	35	40	50	43	43	18
	Present	15	10	0	5	7	31
	Total Incidence	15	10	0	5	7	31
Mineralization	NAD	38	48	43	41	36	42
	Minimal	3	1	2	2	5	3
	Mild	5	0	1	2	4	0
	Moderate	3	1	1	3	5	1
	Marked	1	0	3	0	0	3
	Total Incidence	12	2	7	7	14	7
Pyelitis	NAD	50	48	45	46	48	49
	Minimal	0	0	1	0	1	0
	Mild	0	1	0	0	0	0
	Moderate	0	1	2	0	1	0
	Marked	0	0	2	2	0	0
	Total Incidence	0	2	5	2	2	0

[a]No abnormalities detected.

The clinical pathology findings did not show any changes that might be related to renal toxicity (Table 12). The analyses of blood and serum would detect only rather severe renal disease. Urinalysis, which would detect more subtle renal effects, was not conducted.

Table 12
Hematology and Clinical Chemistry in Serial-Sacrifice Rats After DU(Ti) Implants
(range of values)

	Pre	10 Days	1 Month	2 Months	6 Months	12 Months	12 Months
	Sham	DU(Ti)	DU(Ti)	DU(Ti)	DU(Ti)	DU(Ti)	Sham
Number in Group	4	4	4	4	4	5	5
Hematology							
WBC[a] (10^3/cmm)	5.0–6.8	3.9–5.1	2.9–7.2	4.2–5.4	3.6–6.1	2.5–5.0	2.9–7.0
RBC[b] (10^6/cmm)	9.02–9.27	8.9–11.1	8.96–10.25	9.14–9.47	6.39–8.3	7.57–8.05	6.49–7.75
HCT[c] (%)	45.3–50.2	45.6–51.6	41.9–50	39.9–46.7	34.3–42.5	39–46.1	36.1–43.5
Clinical Chemistry							
ALB[d] (g/dL)	3.6–3.8	3.5–3.9	3.5–3.7	3.4–3.6	2.9–3.3	2.2–2.8	2.5–2.7
ALP[e] (IU/L)	64–124	68–86	0–4	61–66	68–114	47–133	30–131
CPK[f] (IU/L)	109–808	139–204	170–1330	228–880	294–818	47–184	192–797
Calcium (mg/dL)	10.4–13.4	10.2–11.1	–	10.5–10.6	10.5–11.1	9.6–11.4	10.1–10.7
Creatinine (mg/dL)	0.6–0.7	0.6–0.7	0.8–1.1	0.7–1.1	0.7–0.8	0.8–1.1	0.7–1.0
Urea nitrogen (mg/dL)	20–26	22–25	23–28	19–21	17–25	23–31	21–30
Phosphorus (mg/dL)	8.4–9.8	7.3–9.3	6.3–8.9	6.4–8.0	6.1–7.7	5.0–6.5	5.2–6.7
Total protein (g/dL)	6.1–6.9	6.2–6.8	6.2–7.0	6.2–6.4	6.8–7.1	6.1–6.6	6.0–6.7

[a]White blood cells.
[b]Red blood cells.
[c]Hematocrit.
[d]Albumin.
[e]Alkaline phospatase.
[f]Creatine phosphokinase.

Histopathology of the kidneys from the rats in the serial sacrifice study showed no lesions at 10 days, but a moderate to marked tubular regeneration was evident one month after implantation in three of the four rats (Table 13).

Table 13
Renal Lesions in Serial Sacrifice Rats

Rat No.	DPI (days)	Tubular Regener-ation	Tubular Casts	Nephro-pathy	Mineral-ization	U Conc. Kd (µg U/g Kd)	Comment
T031	10	0	0	0	0	1.16	
T038	10	0	0	0	0	3.17	
T039	10	0	0	0	0	1.11	
T043	10	0	0	0	0	0.35	
T030	33	4	0	0	0	6.46	
T037	33	4	0	0	0	11.62	
T048	33	3	1	0	0	7.1	
T058	33	0	0	0	0	ND	

Table 13
Renal Lesions in Serial Sacrifice Rats (concluded)

Rat No.	DPI (days)	Tubular Regener-ation	Tubular Casts	Nephro-pathy	Mineral-ization	U Conc. Kd (µg U/g Kd)	Comment
T029	65	1	0	0	0	12.09	
T044	65	1	0	0	0	9.35	
T047	65	0	0	0	0	7.57	
T054	65	1	0	0	0	6.43	
T028	127	0	0	0	0	2.07	
T032	127	0	0	0	0	13.62	
T045	127	1	0	0	0	8.46	
T057	127	0	0	0	0	ND	
T024	186	0	0	1	0	12.72	
T025	186	0	0	0	0	6.33	
T041	186	2	0	0	0	15.71	
T053	186	0	0	1	0	8.89	
T050	276	0	0	1	1	5.07	Died
T023	278	0	0	3	0	9.33	
T036	278	0	0	1	0	14.33	
T051	278	0	0	0	0	8.24	
T056	278	0	0	1	0	3.36	
T033	350	0	0	2	0	4.72	M Sac
T027	368	0	0	1	0	3.29	
T035	368	0	0	1	0	2.38	
T042	368	0	0	1	0	4.42	
T046	368	0	0	1	0	4.38	
T049	368	0	0	2	0	6.95	
T034	379	0	0	1	0	3.16	M Sac
T026	558	0	0	3	0	8.59	
T040	558	0	0	3	0	1.29	
T052	558	0	0	2	0	10.43	
T055	558	0	0	3	0	6.46	

0 = no lesion; 1 = minimal; 2 = mild; 3 = moderate; 4 = marked; NA = Not Done

The lesion was characterized by basophilia, high nuclear cytoplasmic ratio, and occasional karyomegaly of the tubular epithelium (Figure 29). It was distinguished from the early changes of nephropathy by the lack of thickening of the basement membrane, lack of inflammatory cell infiltrates, and its more extensive nature. Much of the

epithelium of all segments of the proximal tubules was involved; the distal tubules were largely spared. The lesion was graded on the number of tubules involved. Very occasionally granular casts and individual epithelial cells undergoing apoptosis were seen in the tubules indicative of a tubular necrosis. Only minimal tubular regenerative changes were seen in three of the four rats at 2 months and in one of four rats at 4 months. At later times, no changes were seen in the tubular epithelium that could be distinguished from nephropathy.

3. Discussion

These findings of a rapid increase and slow decline of the daily U excretion rate and the concentration of U in the kidney indicate that an equilibrium is reached in the absorption in the blood and excretion of U within a week to a few months after implantation. Table 14 shows that the content of U in the kidney had a better correlation with surface area than mass, as might be expected. However, the U concentration in the rats of the 5.0 × 5.0 mm group was not as great as expected and similar to the 2.5 × 2.5 mm group. This may be due to the intense capsule-forming lesions around the implants which may have resulted in a saturation of dissolution or transport mechanisms.

Table 14
U Content in Kidney of Groups of Rats Implanted with DU(Ti) Fragments

DU(Ti) Fragment Group	Mean U/ Kidney (μg)	Ratio of Kidney U Content	Ratio of Fragment Surface Area	Ratio of Fragment Mass
2.0 × 1.0 mm diam.	6.7	1	1	1
2.5 × 2.5 mm	24.4	3.6	3.5	5.8
5.0 × 5.0 mm	28.0	4.2	10	40

The U in the kidney reached high concentrations within a month after implantation. The temporal patterns of concentrations were similar to those reported after the implantation of 20 DU(Ti) pellets (2.0 mm × 1.0 mm diam.) (Pellmar *et al.*, 1999). The peak concentration, however, was greater after the implant of four 2.5 × 2.5 × 1.5 mm fragments of DU(Ti) (10.9 μg/g vs. 6.9 μg/g) for 20 pellets. The mass of the four fragments was somewhat greater than the 20 pellets (698 mg vs. 595 mg.), but the surface area was less (110 mm^2 vs. 157 mm^2).

The concentrations of U in the kidney in these two studies is much greater than that noted in most studies of U administered in other forms and by other routes. For example, in a study of renal toxicity in rats, the highest U burdens were 5.6 μg/g, which were achieved 3 days after five biweekly injections of 120 μg and three injections of 240 μg of uranyl fluoride (Diamond *et al.*, 1989). In a study of inhaled U, the highest renal burden after 6-months exposure to 5 mg/m^3 was 2.5 μg/g (Leach *et al.*, 1973). However, studies of rats that were fed UO_2F_2 or $UO_2(NO_3)_2$, soluble forms of U, showed that renal concentrations of U can achieve the 15 to 25 μg/g range (Hodge, 1953).

Renal lesions consisting of tubular regeneration and scant other evidence of prior tubular necrosis were seen in rats sacrificed one and two months after exposure with renal burdens of about 8 μg U/g kidney. The severity of these lesions was not as great as anticipated, based on earlier reports. A concentration of 3 μg/g kidney has been considered the concentration for limiting renal toxicity from U (Leggett, 1989) and, in the kidney of rats with acute lethal doses of uranyl ion, 40 to 50 μg U/g kidney was found (Hodge, 1953). However, there is considerable variability in U renal toxicity with species, chemical form, and exposure pattern. For example, renal lesions were severe and extensive in rats injected with uranyl fluoride and achieving U concentrations of 3.4 to 5.6 μg/g and were obvious in rats with concentrations of 0.7–1.4 μg/g. These lesions resulted in functional abnormalities, but the rats recovered within 35 days (Diamond *et al.*, 1989). Renal concentrations of 0.1–0.4 μg/g in dogs, rabbits, and rats after inhalation of UF_6 or UCl_4 over several months caused occasional mild renal injury (Hodge, 1953).

These results indicated that the prolonged dissolution of U from a DU(Ti) fragment led to persistent concentrations of U in the kidney. The renal concentration seemed to be related to the size of the fragment at the lower doses, as was shown by the studies with various numbers of DU(Ti) pellets (Pellmar *et al.*, 1999). However, the two large fragments (2.5 × 2.5 mm and 5.0 × 5.0 mm) resulted in similar renal concentrations of U over the lifetime of the rats. Interestingly, the U kidney concentrations showed an equilibrium or saturation in type of dynamic compared to an ever-increasing U kidney burden. This latter behavior would occur if the retention of U in the kidney was more avid or prolonged and would result in a poorer prognosis for renal health.

KEY RESEACH ACCOMPLISHMENTS

1. DU (0.75 Ti) fragments, embedded in the muscles of rats, caused localized soft tissue tumors. The incidence of tumors was significantly greater than that of similarly sized Ta metal fragments indicating that the response was not a nonspecific foreign-body response typically seen in rats.
2. The incidence of DU(Ti)-induced tumors is correlated to the surface radioactivity of the fragments. The significance of this correlation for the carcinogenic mechanisms remains to be determined.
3. DU(Ti) rapidly corroded in the tissues of rats causing a marked chronic inflammatory reaction and capsule formation, a finding that may relate to the mechanism of carcinogenesis.
4. Radiography can illustrate the break-up of corroding DU(Ti) fragments and the resultant proliferative tissue reaction, a feature that may be useful in the medical evaluation of individuals wounded with DU(Ti) fragments.
5. Implanted DU(Ti) did not increase tumor incidence other than at the implant sites. The lack of tumors in other tissues is consistent with observations indicating a lack of U carcinogenicity in humans.
6. Renal concentrations of 15–20 μg U/g kidney did not affect life span.
7. Renal excretion of U increased for about 3–6 months, then peaked and slowly declined after exposure to U dissolving from DU(Ti) implants. These findings on the renal concentration and renal excretion indicate that the urinary toxicity of chronic exposure to U may not be as severe as previously indicated.
8. The renal concentration of U could not be related with the daily urinary excretion rate.

REPORTABLE OUTCOMES

Abstracts

Hahn, FF, Guilmette, RA, Hoover MD. Toxicity of Depleted Uranium Fragments in Wistar Rats. Toxicological Sciences 48, 1-S, 333, 1999.

Hahn, FF, Guilmette, RA, Hoover, MD. Toxicity of Depleted Uranium Fragments in Wistar Rats. Health Physics, 78-S, 129, 2000.

Presentations

Hahn, FF. DU Distribution and Carcinogenesis Studies. Armed Forces Radiobiology Research Institute Workshop, "Health Effects of Embedded Depleted Uranium Fragments," Nov 15, 1996.

Hahn, FF. Toxicity of Depleted Uranium Fragments in Wistar Rats. Society of Toxicology Annual Meeting, Seattle, WA, March 1999.

Hahn, FF. Two Year Carcinogenesis Study in Rats with Implanted DU Pellets. Baltimore VA Medical Center, March 17, 2000.

Hahn, FF. Toxicity of Depleted Uranium Fragments in Wistar Rats. Health Physics Society Annual Meeting, Denver, CO, June 25–29, 2000.

Hahn, FF. Depleted Uranium Fragments Cause Soft Tissues Sarcomas in the Muscles of Rats. Conference on Illnesses Among Gulf War Veterans: A Decade of Scientific Research. Alexandria, VA, January 24–26, 2001.

Publications

Hahn, FF, Lundgren, DL, Hoover, MD, Guilmette, RA. DU Distribution and Carcinogenesis Studies. In "Health Effects of Embedded Depleted Uranium Fragments" (Livengood, OR, ed.), An Armed Forces Radiobiology Institute Workshop, 1996, AFFRI Special Report Publication 98-3, National Technical Information Service, 1998.

Hahn, FF, Guilmette, RA, Hoover MD. Implanted Depleted Uranium Fragments Cause Soft Tissue Sarcomas in the Muscles of Rats. Environmental Health Perspectives (submitted and reviewed).

Funding Applied for Based on Work Supported by this Award

Hahn, FF. "Implant-Associated Carcinogenesis in Heterozygous $p53^{+/-}$ Mice: Pilot Study," to U.S. Army Medical Research Acquisition Activity, August 12, 1999.

Hahn, FF. "Depleted Uranium Fragment Carcinogenicity: Extrapolation of Findings in Rodents to Man," to US Army Medical Research Acquisition Activity, July 25, 2000.

CONCLUSIONS

Carcinogenesis

DU(Ti) fragments implanted in the muscles of rats cause localized soft tissue sarcomas. The effect is related to the radioactive or corrosive properties of the DU(Ti) and is not a nonspecific response of the rat to an implanted foreign body. DU(Ti) fragments in the muscles of rats corrode rapidly, creating a distinctive radiographic pattern. Capsules are formed around the corroding DU(Ti) fragments, and it is from these capsules that the sarcomas arise. The initial stages of the neoplasm result in an erosion of the DU(Ti) fragments that is detectable with radiography. This feature may be useful in the clinical evaluation of individuals wounded with DU(Ti) fragments.

Renal Toxicity

Rats with DU(Ti) implants can achieve renal burdens of U that are in excess of 3 μg/g kidney. In spite of these high renal concentrations, renal toxicity did not shorten the life span of the animals. The daily urinary excretion peaked at 6 months after exposure and then slowly declined. These findings indicate that the chronic renal toxicity of U may not be as severe as previously indicated. The daily urinary excretion rate of U as a fraction of the implanted fragment is about 10^{-5} in rats.

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APPENDIX A

Acronym and Symbol Definition

ABDAC	=	Alkylbenzyldimethylammonium chloride
AFFRI	=	Armed Forces Radiobiology Research Institute
ALB	=	Albumin
ALP	=	Alkaline phosphatase
ANOVA	=	Analysis of variance
COD	=	Cause of death
CPK	=	Creatinine phosphokinase
DPI	=	Days post-implantation
DTPA	=	Diethylenetriaminepentaacetic acid
DU	=	Depleted uranium metal
DU(Ti)	=	Depleted uranium + 0.75% titanium metal alloy
HCT	=	Hematocrit
H&E	=	Hematoxylin and eosin
INC	=	Incidental
LN	=	Lymph node
LRRI	=	Lovelace Respiratory Research Institute
M Sac	=	Moribund sacrifice
MFH	=	Malignant fibrous histiocytoma
NAD	=	No abnormalities detected
NOS	=	Not otherwise specified
RBC	=	Red blood cells
SUF	=	Synthetic serum ultrafiltrate
Ta	=	Tantalum metal
U	=	Uranium
WBC	=	White blood count

APPENDIX B
Figures

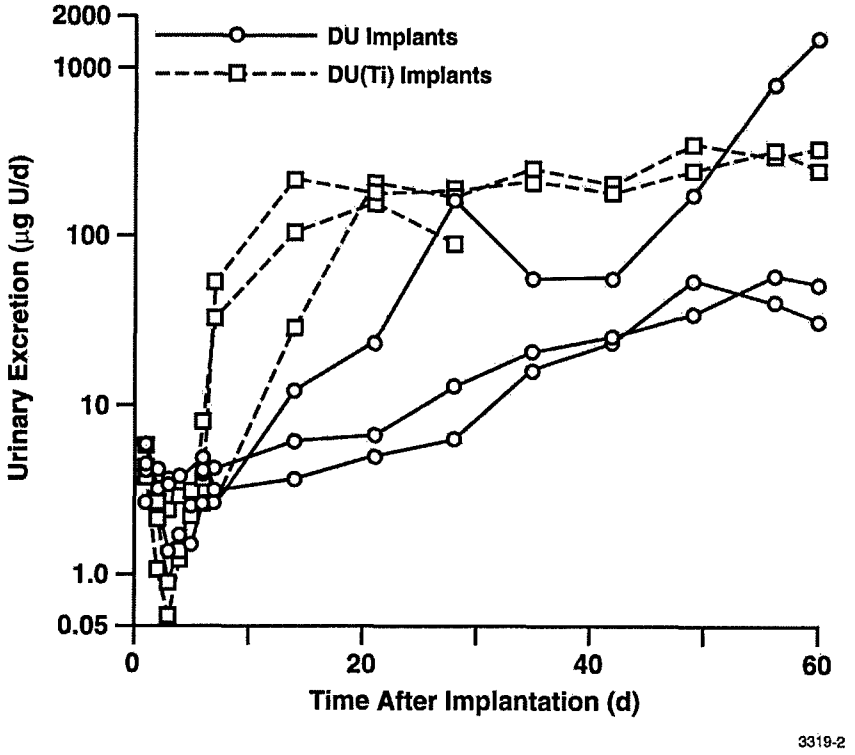

Figure 1. Urinary excretion of U in individual rats with DU or DU(Ti) implants.

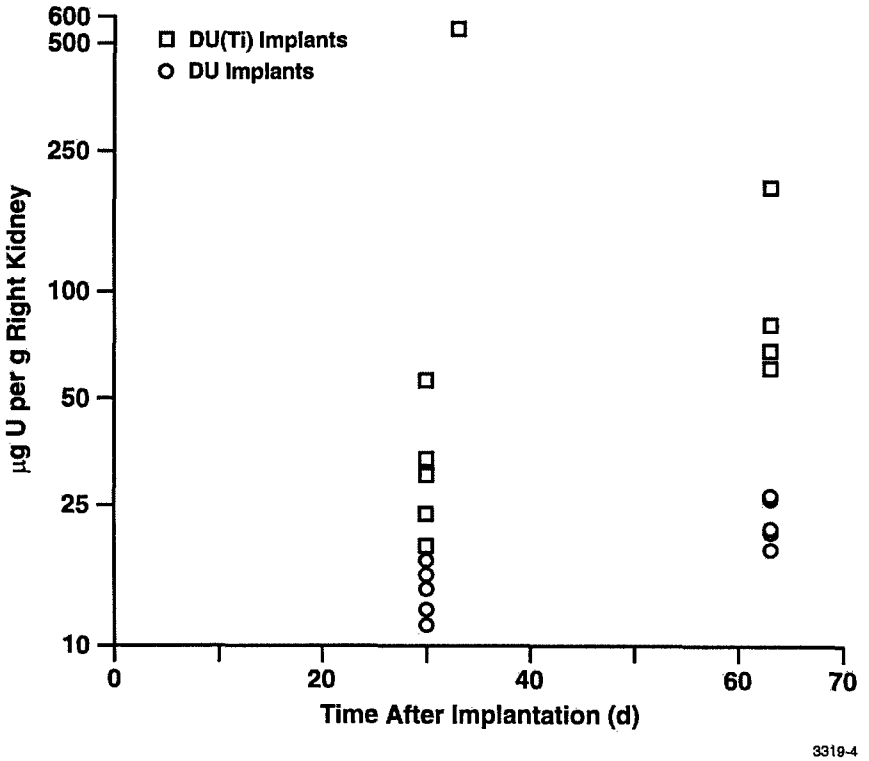

Figure 2. Renal concentration of U in rats with DU or DU(Ti) implants.

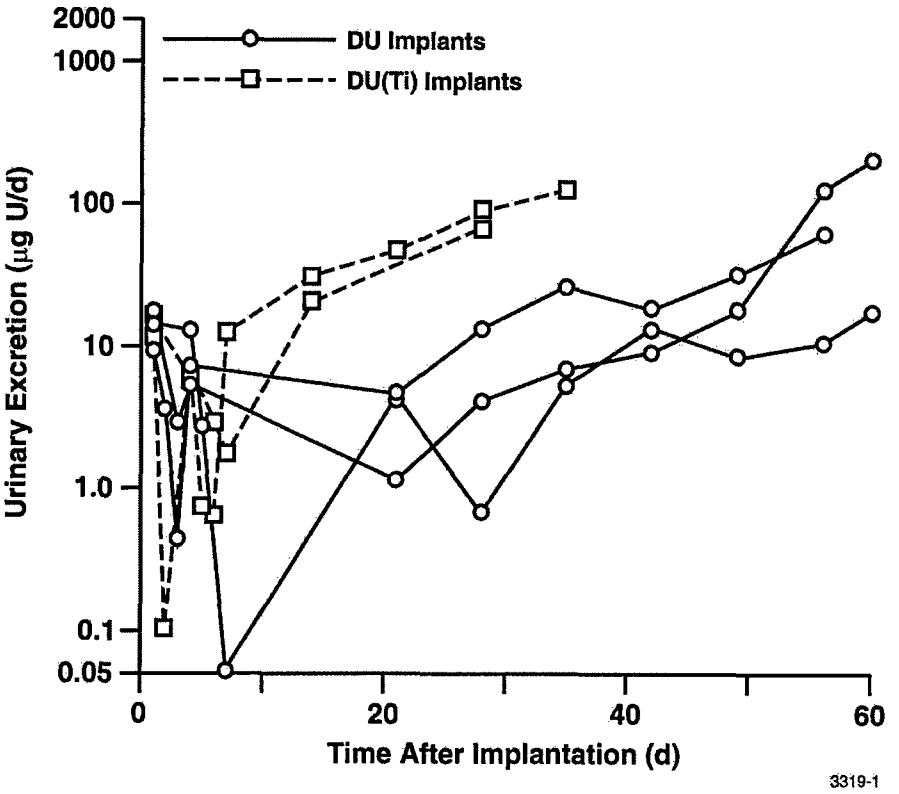

Figure 3. Urinary excretion of U in individual mice with DU or DU(Ti) implants.

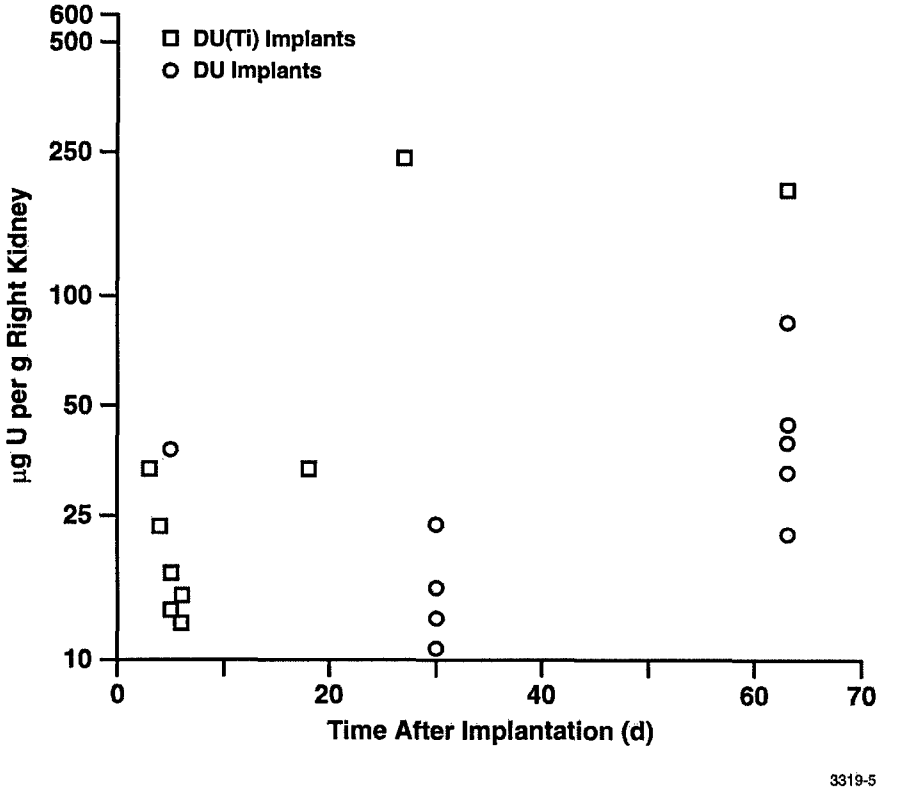

Figure 4. Renal concentration of U in mice with DU or DU(Ti) implants.

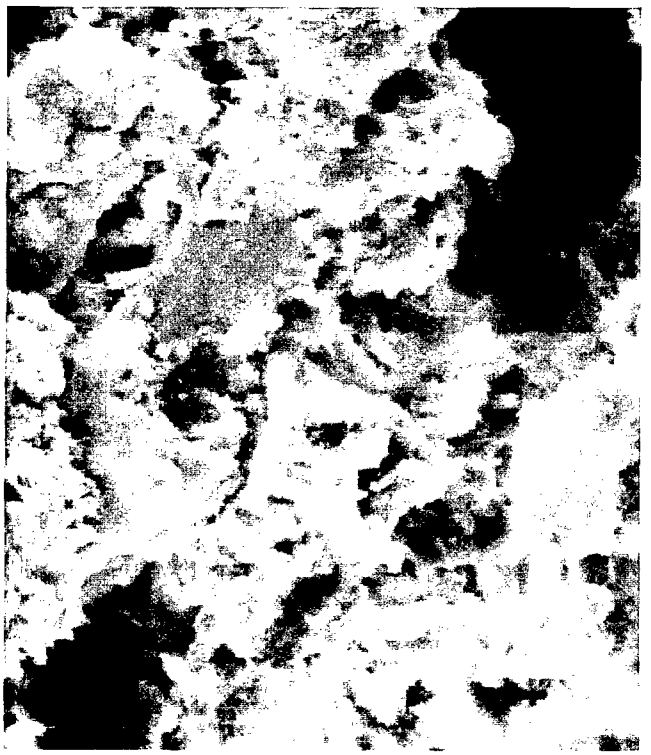

Figure 5. Scanning electron microscopy comparison of DU(Ti) foil (A) 30 days after implantation with control foil before implant (B).

Figure 6. Fibrous capsule around a DU(Ti) implant showing infiltration of chronic inflammatory cells (H&E staining).

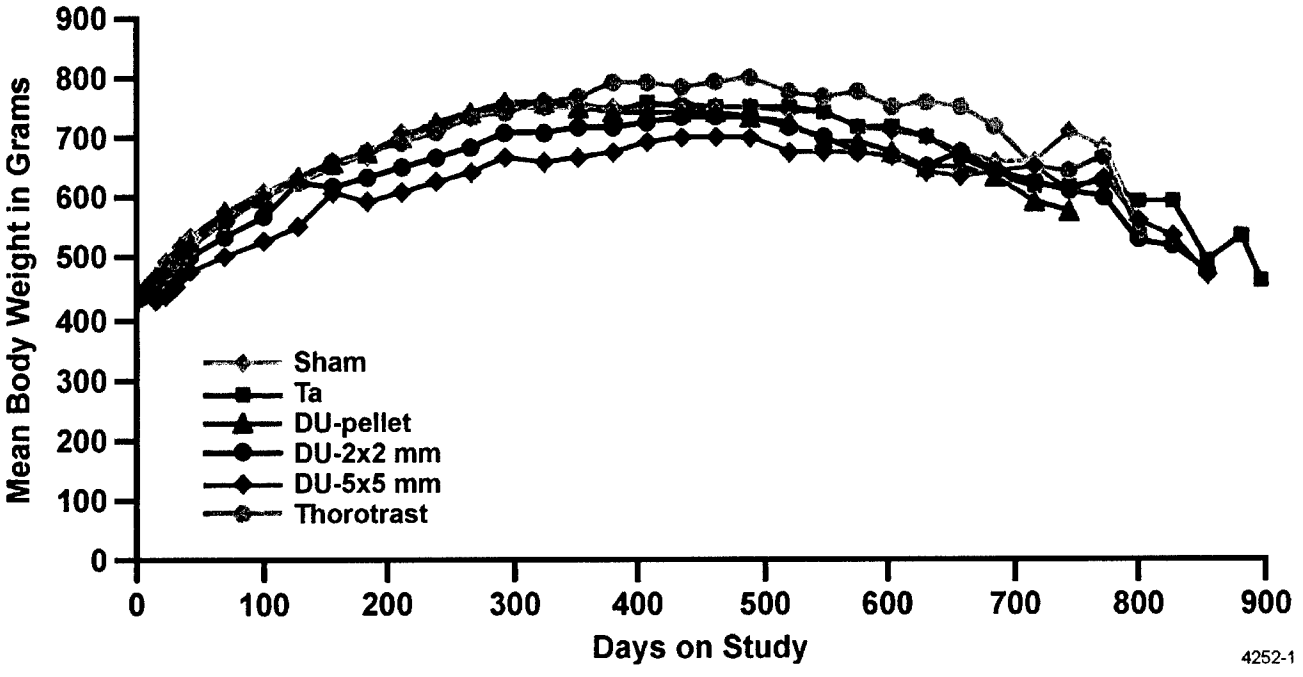

Figure 7. Body weights of rats with DU(Ti) implants and with positive, negative, and surgical-control rats.

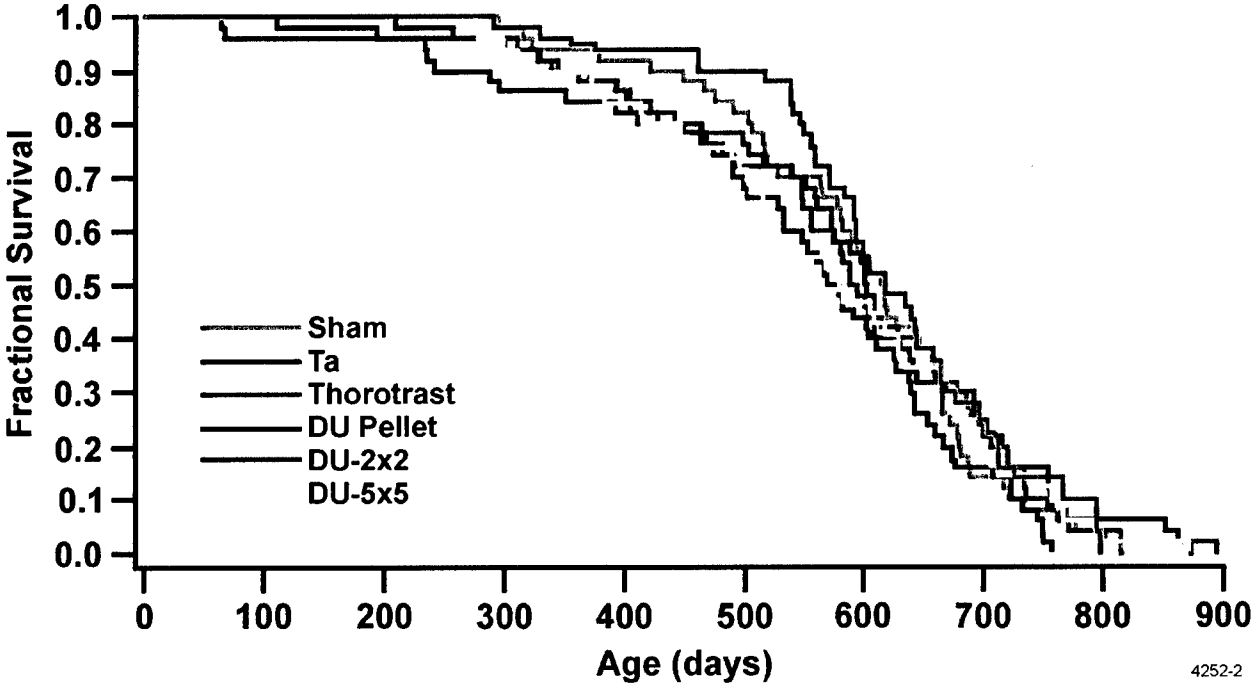

Figure 8. Survival of rats implanted with DU(Ti) compared with positive, negative, and surgical controls.

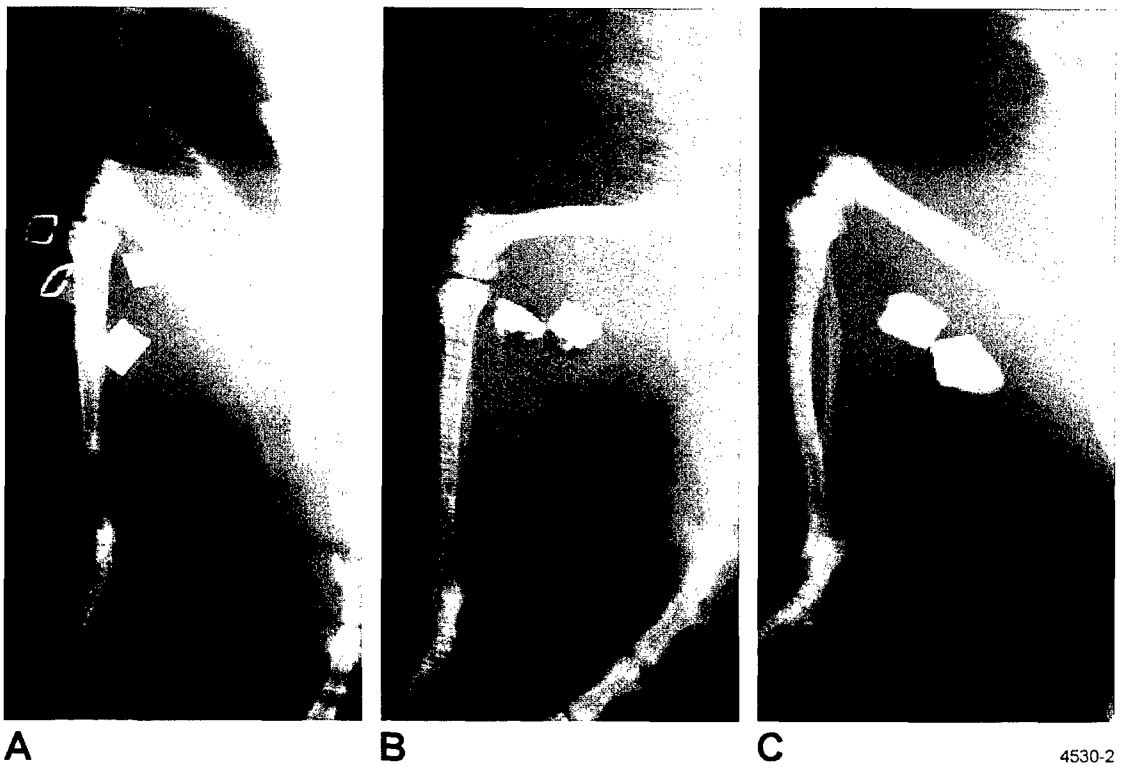

Figure 9. Radiograph of 5.0 × 5.0 × 1.5 mm DU(Ti) fragments in rat R097-6040: (A) on day of implantation - radio-opaque wound clips visible on the skin; (B) 3 weeks after implantation; (C) 1 year after implantation.

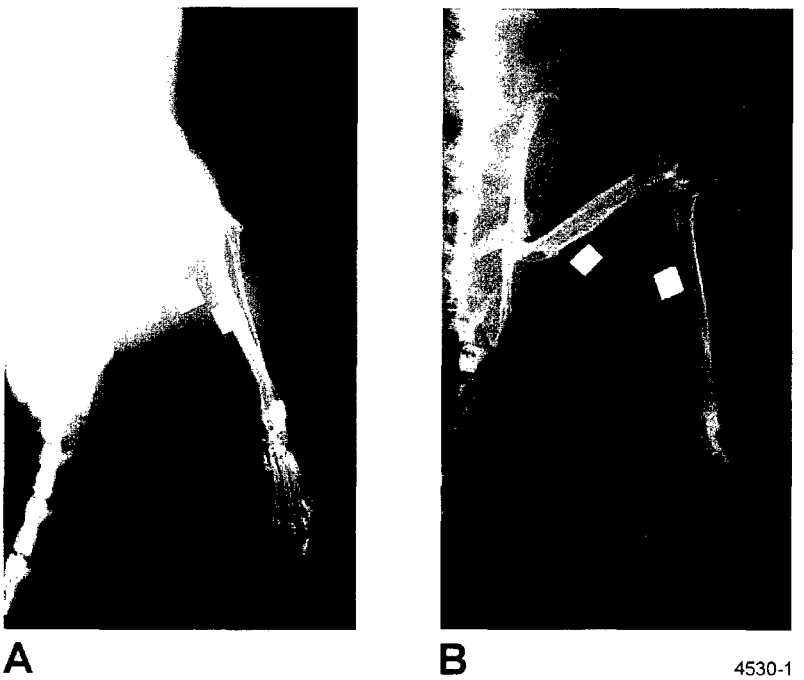

Figure 10. Radiograph of 5.0 × 5.0 × 1.1 mm Ta fragments: (A) 10 weeks after implantation (rat B020-6024); (B) 2 years after implantation (rat N026-6036).

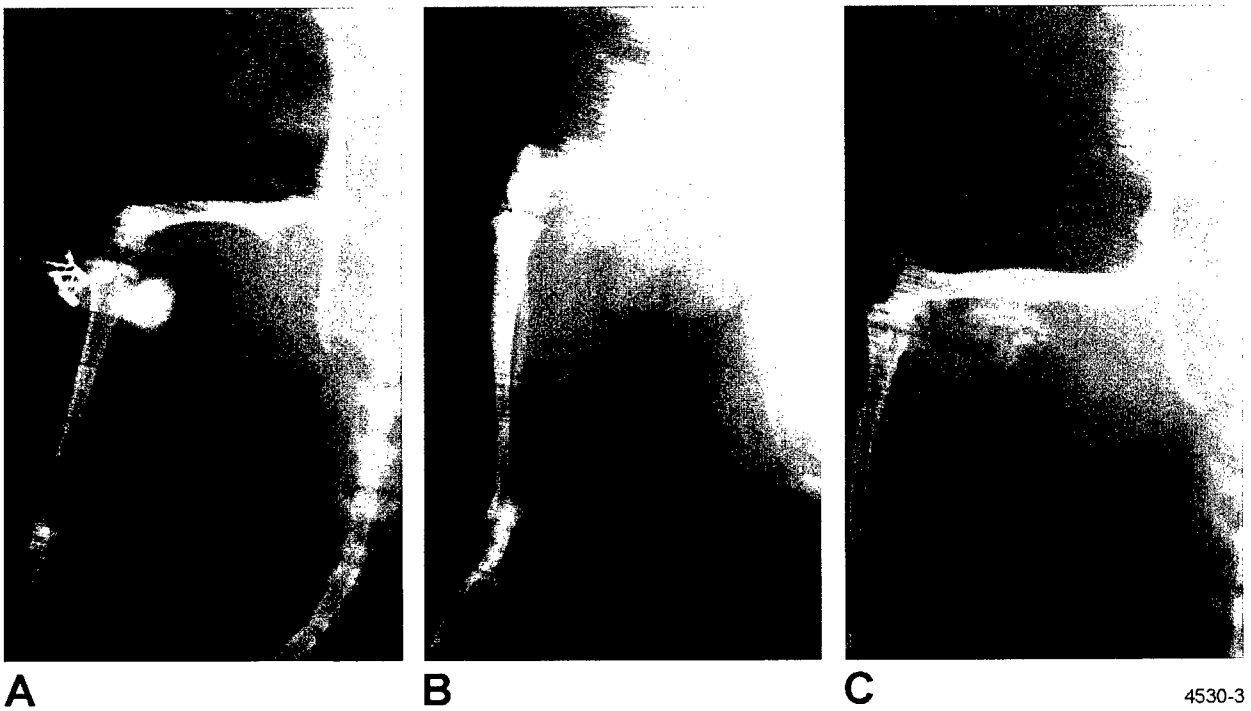

Figure 11. Radiograph of Thorotrast® injection in rat I047-6037: (A) on day of injection; (B) 4 weeks after injection; (C) 1.5 years after injection.

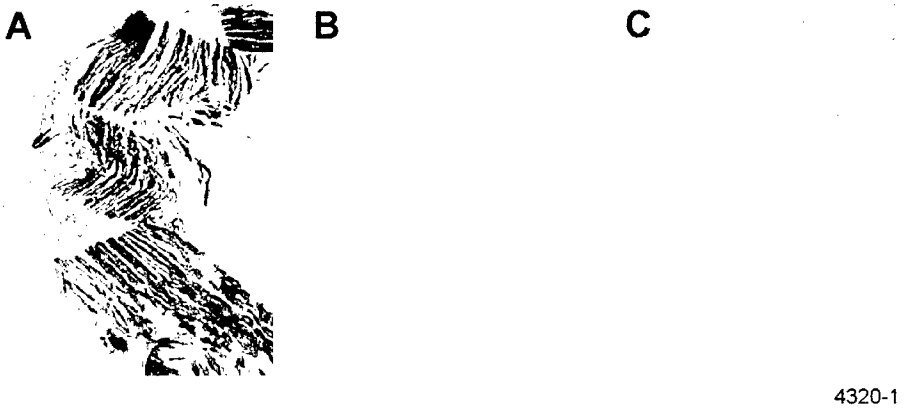

Figure 12. (A) DU(Ti) fragment: Black shards of corroded DU(Ti) lined the thick cellular fibrotic capsule surrounding the fragment 520 days after implantation. (B) Ta fragment: A thin acellular fibrotic capsule with no metal pieces present 603 days after implantation. (C) Thorotrast® injection: No capsule or inflammation; Thorotrast®-laden macrophages infiltrated skeletal muscle 792 days after injection.

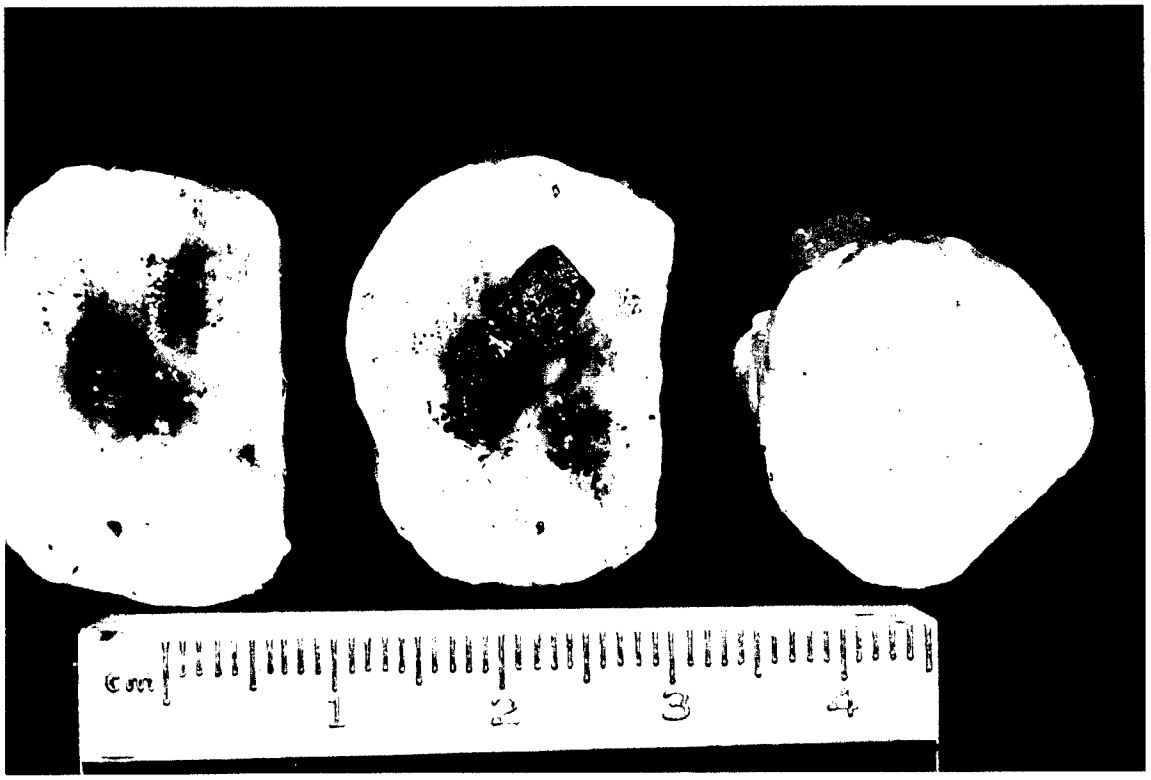

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Figure 13. Photograph of sectioned, localized sarcoma around 5.0 × 5.0 mm DU(Ti) fragment showing fragment and black corrosion particles (rat R100-6040).

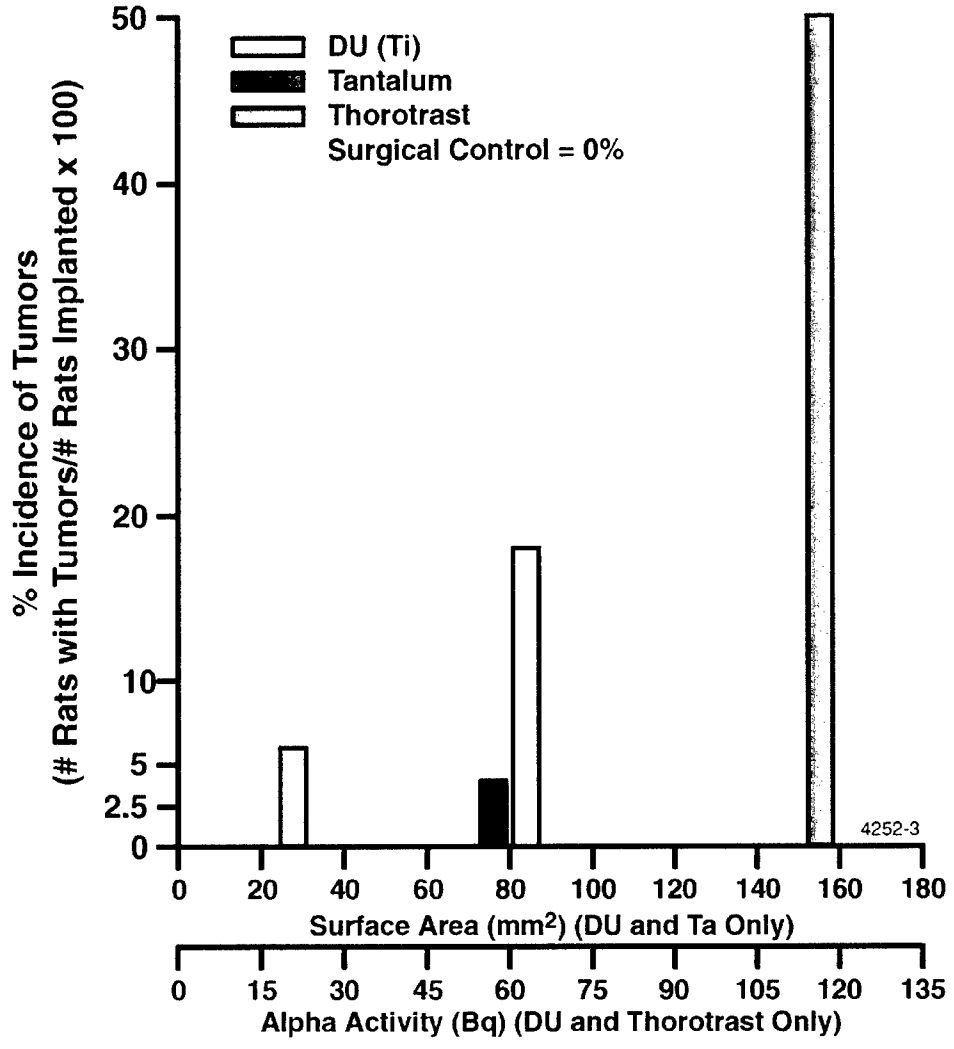

Figure 14. Incidence of soft tissue tumors in rats implanted with DU(Ti), Ta, or Thorotrast®. The incidence of tumors in rats with 5.0 × 5.0 mm DU(Ti) fragments was significantly increased over the incidence in rats with Ta fragments of the same size. The positive control, Thorotrast®, produced the highest incidence of tumors. Thorotrast® > DU(Ti) 5.0 × 5.0 mm, $p = 0.0014$; DU(Ti) 5.0 × 5.0 mm > sham control, $p = 0.0012$; DU(Ti) 5.0 × 5.0 mm > Ta, $p = 0.028$; Fischer's exact test.

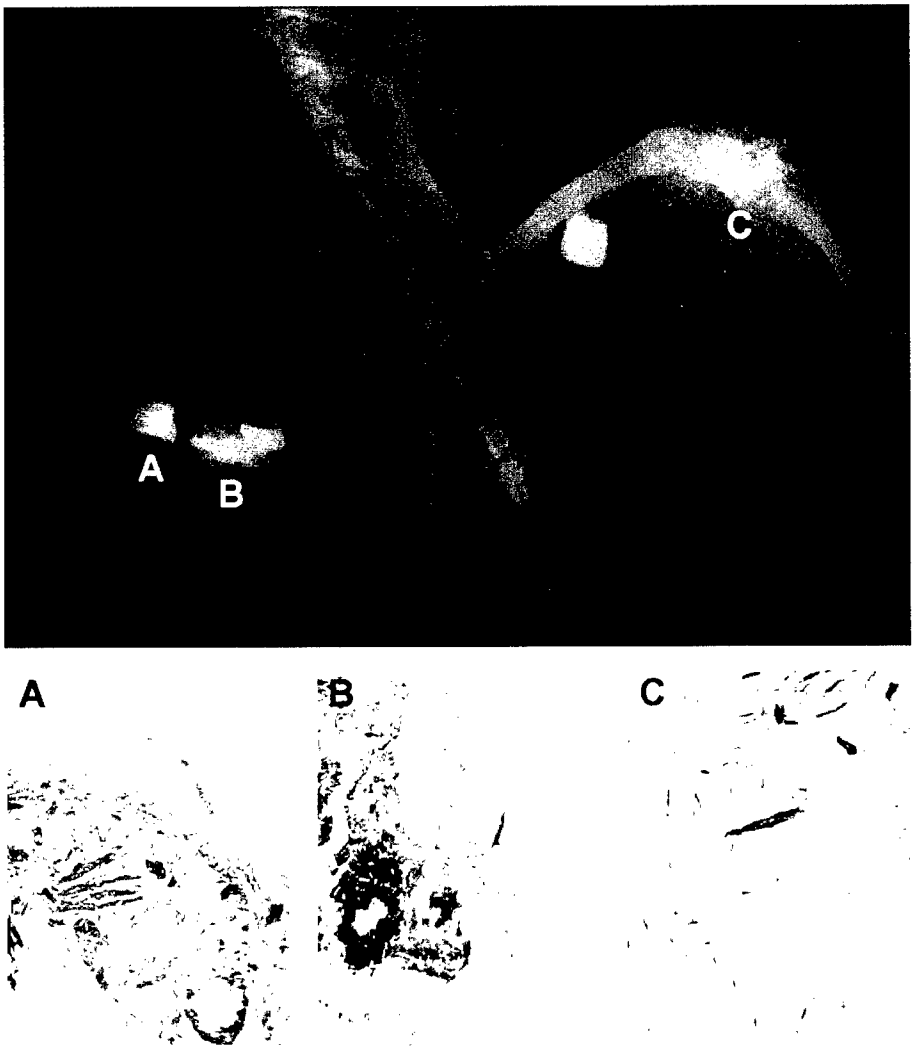

Figure 15. Correlation of radiographic appearance with histologic appearance: (A) thick fibrotic capsule with shards of corroded DU(Ti) in lumen; (B) thick cellular capsule lined by squamous metaplasia, particles, and shards of corroded DU(Ti) in wall and lumen; and (C) particles and shards of disintegrated DU(Ti) fragment scattered throughout a soft tissue sarcoma.

Figure 16. DU fragment after 30 days dissolution in SUF solvent.

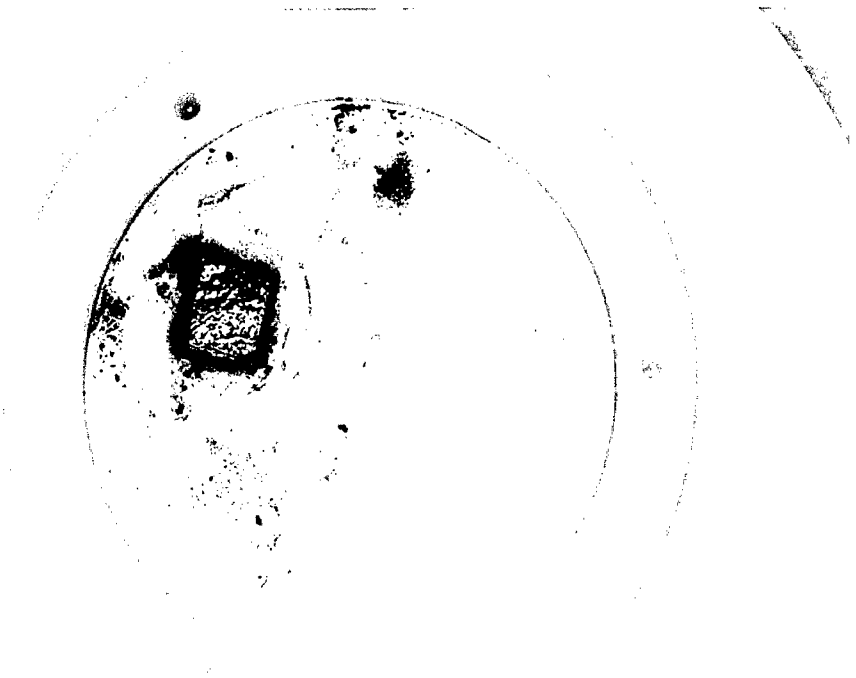

Figure 17. DU(Ti) fragment after 30 days dissolution in SUF solvent.

Figure 18. DU fragment after 30 days dissolution in pH 5 solvent.

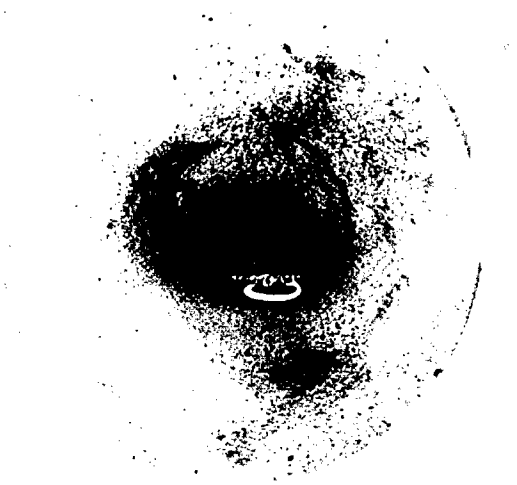

Figure 19. DU(Ti) fragment after 30 days dissolution in pH5 solvent.

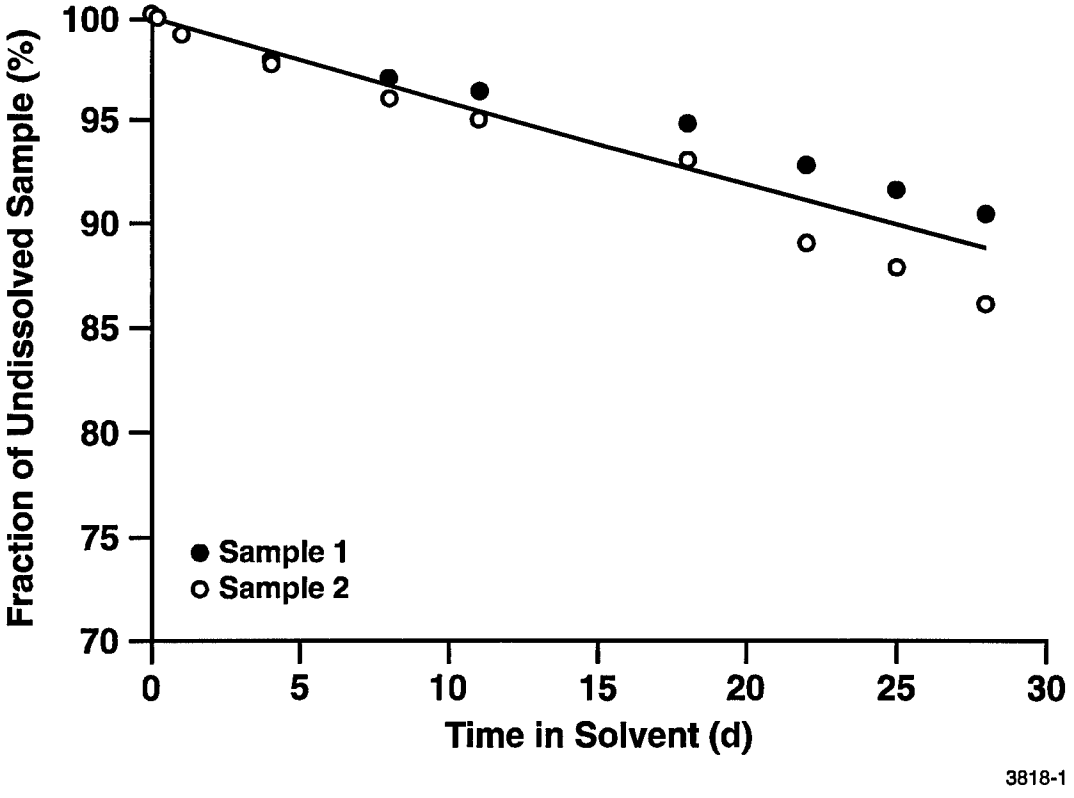

Figure 20. Dissolution of DU in SUF solvent.

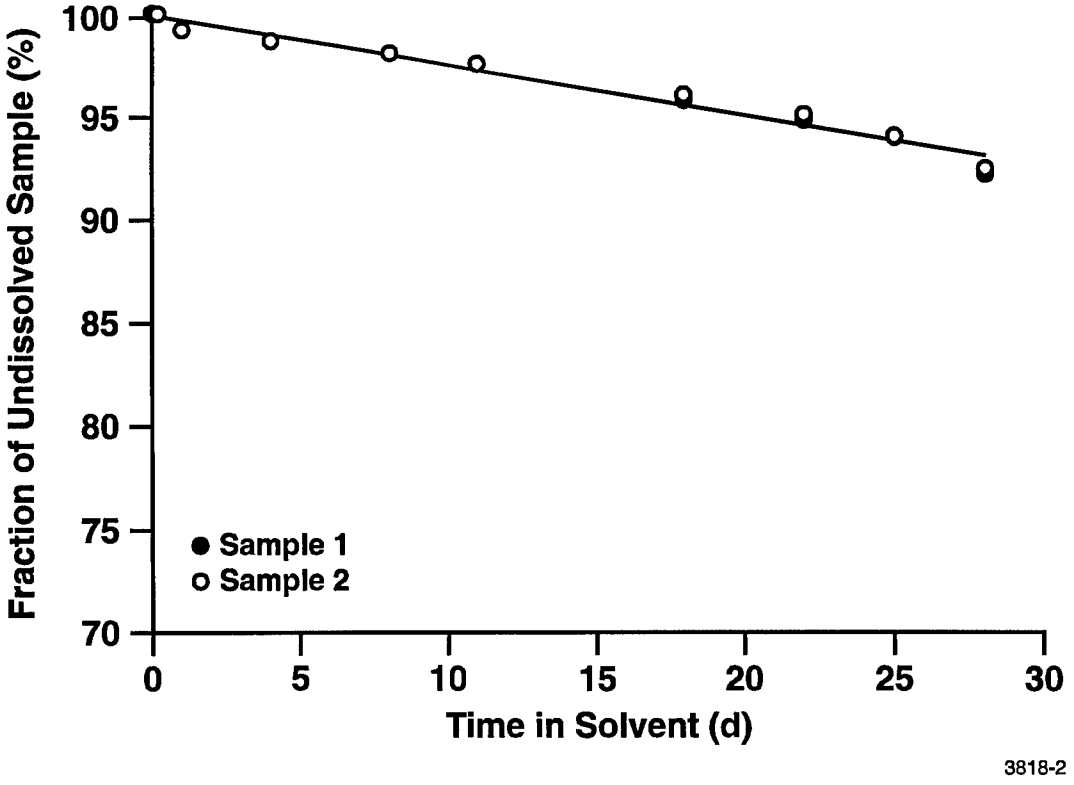

Figure 21. Dissolution of DU(Ti) in SUF solvent.

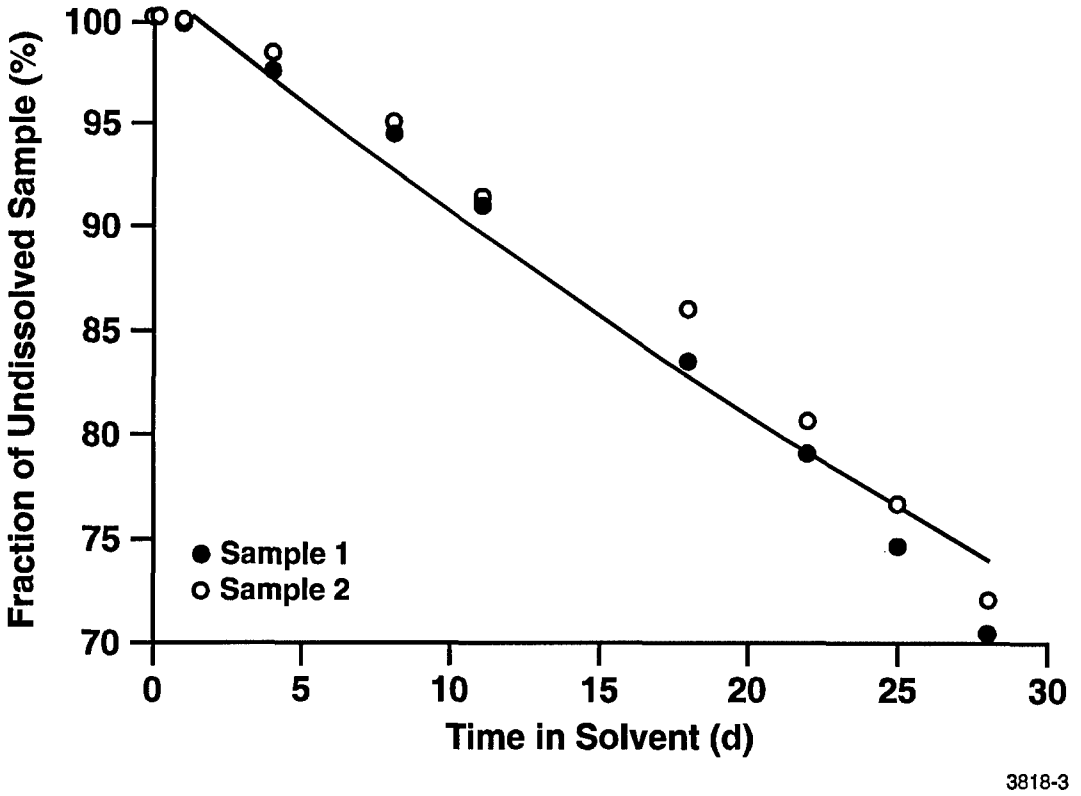

Figure 22. Dissolution of DU in pH 5 solvent.

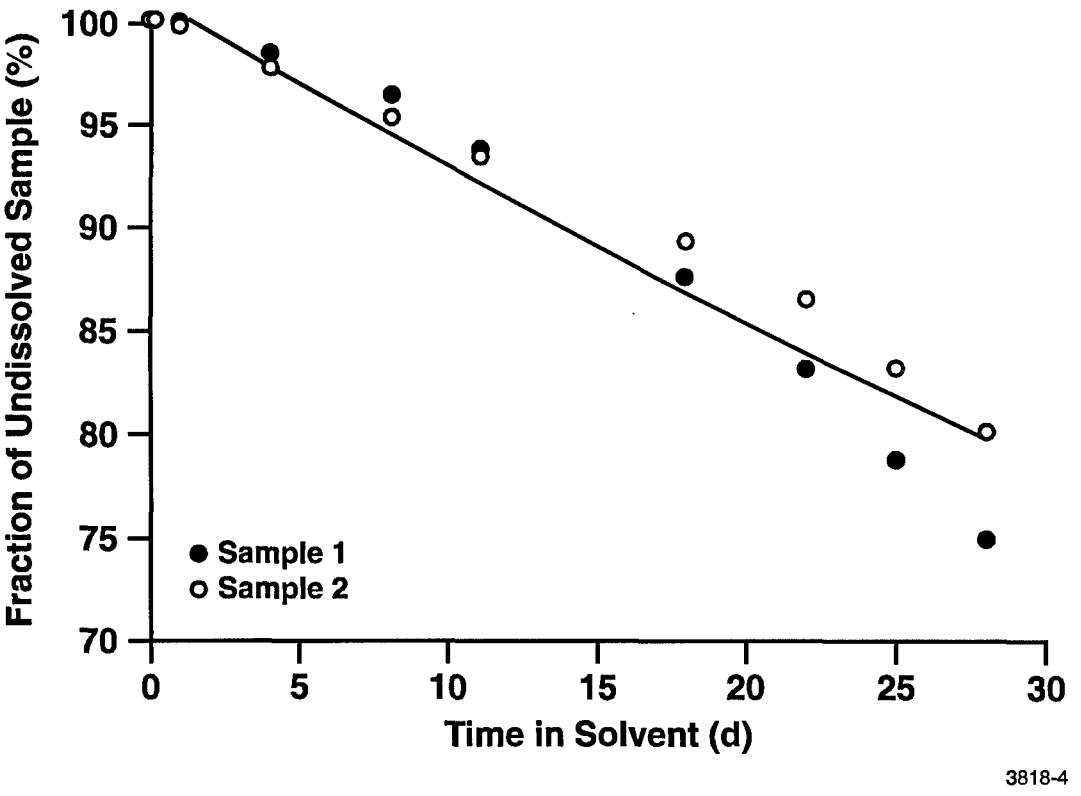

Figure 23. Dissolution of DU(Ti) in pH 5 solvent.

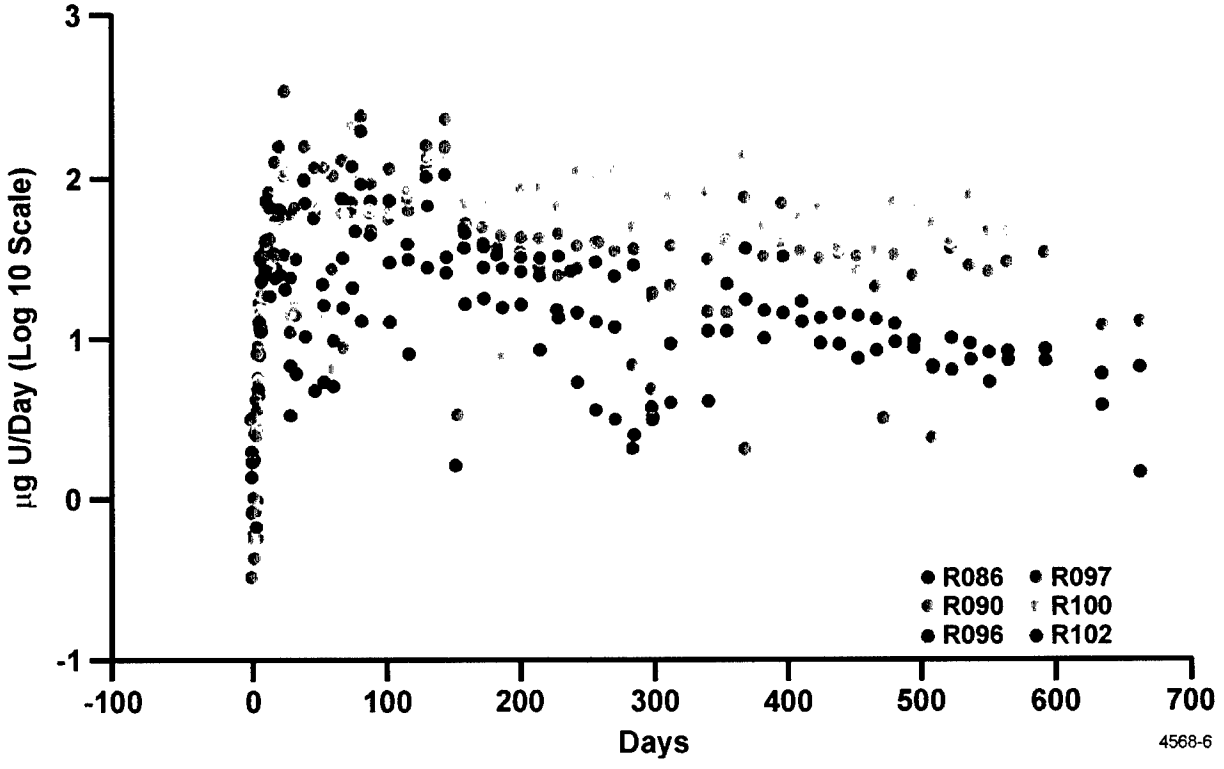

Figure 24. Daily excretion of U in urine of rats implanted with 5.0 × 5.0 mm DU fragments.

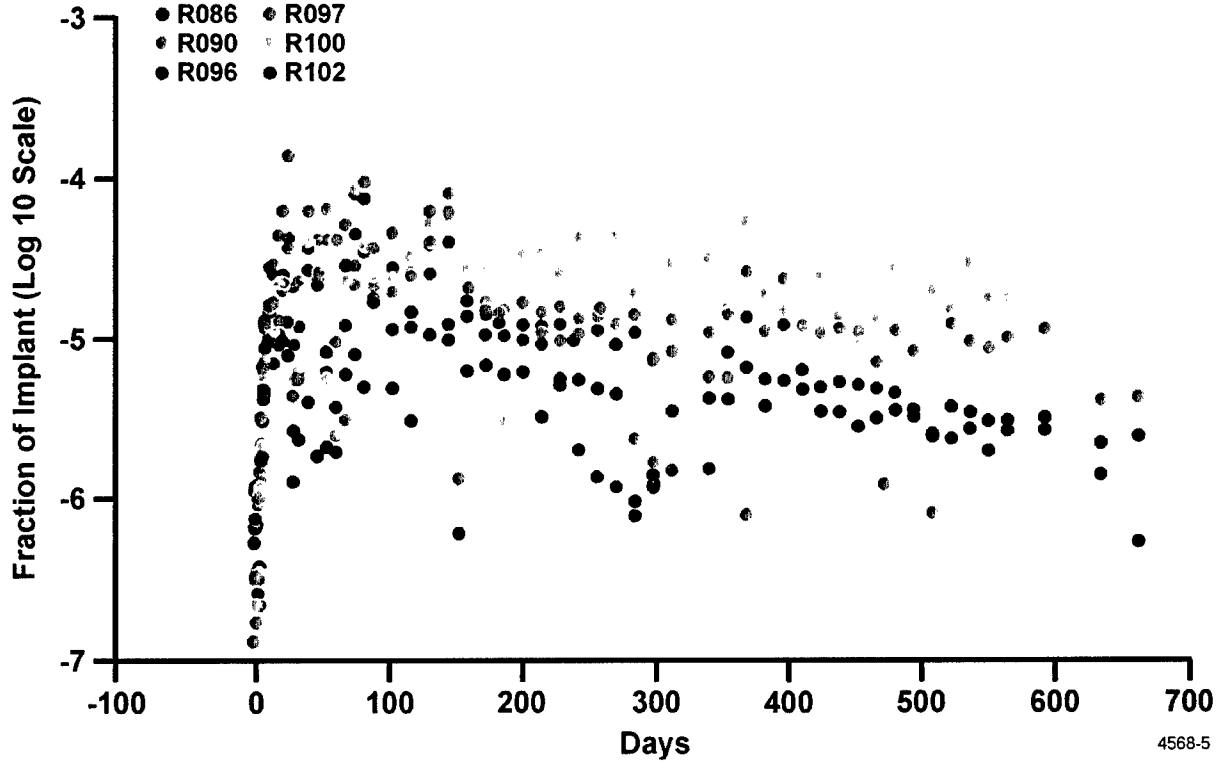

Figure 25. Fraction of DU implant excreted daily in the urine of rats with 5.0 × 5.0 mm DU fragments.

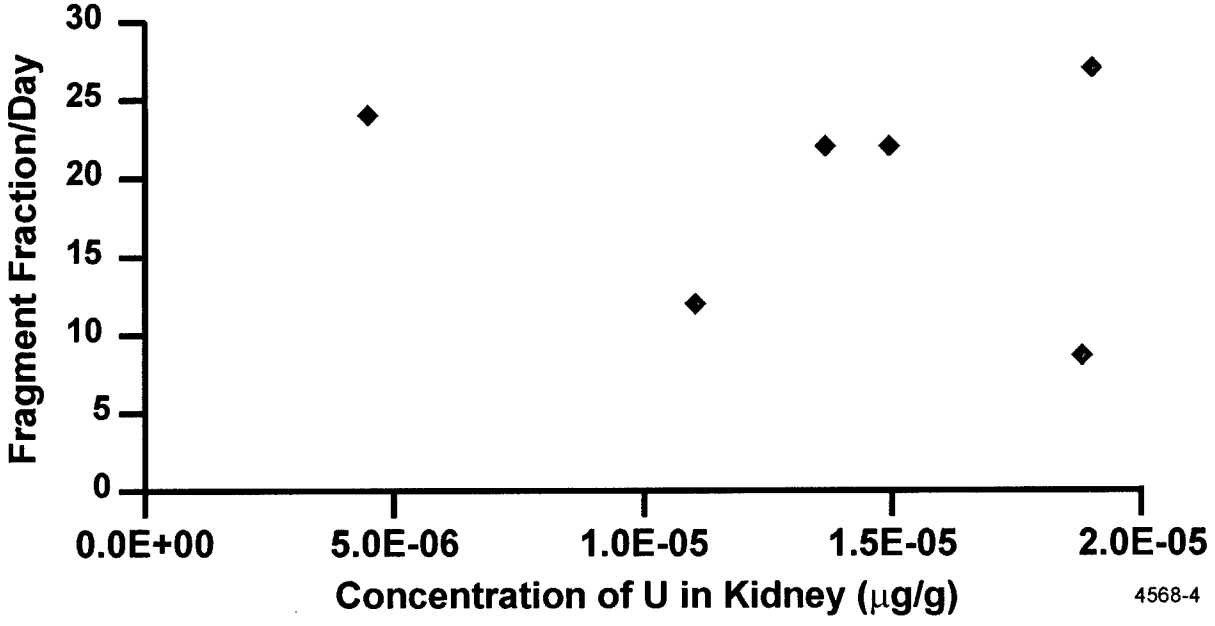

Figure 26. Correlation of fraction of implant excreted daily in urine with renal concentration of U.

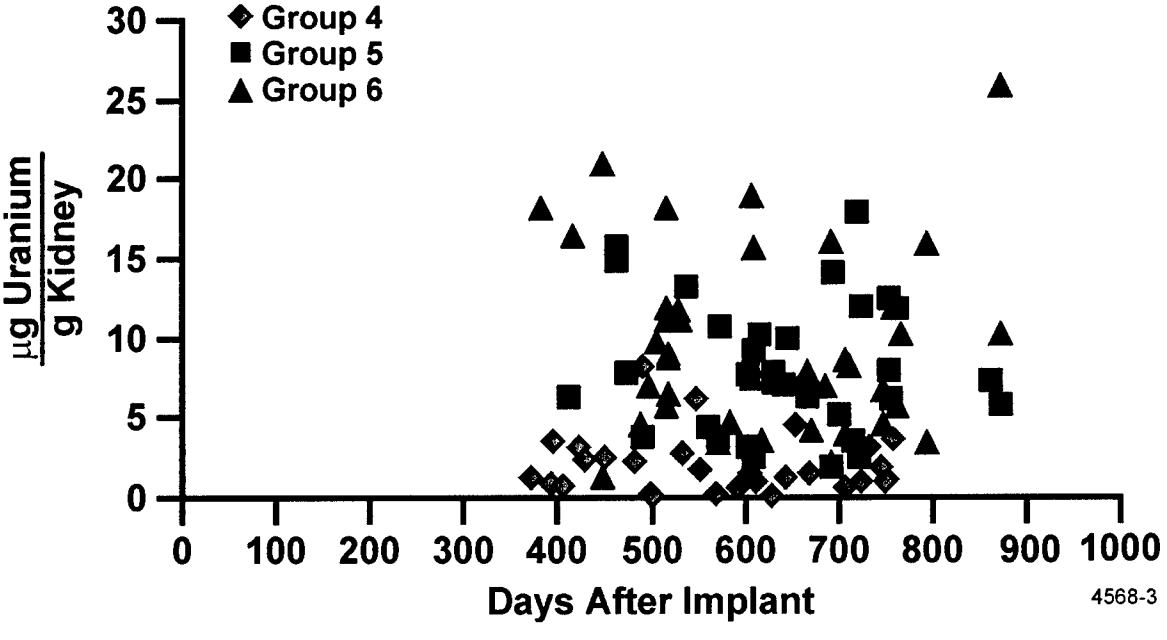

Figure 27. Renal concentration of U in rats that died with implanted DU fragments.

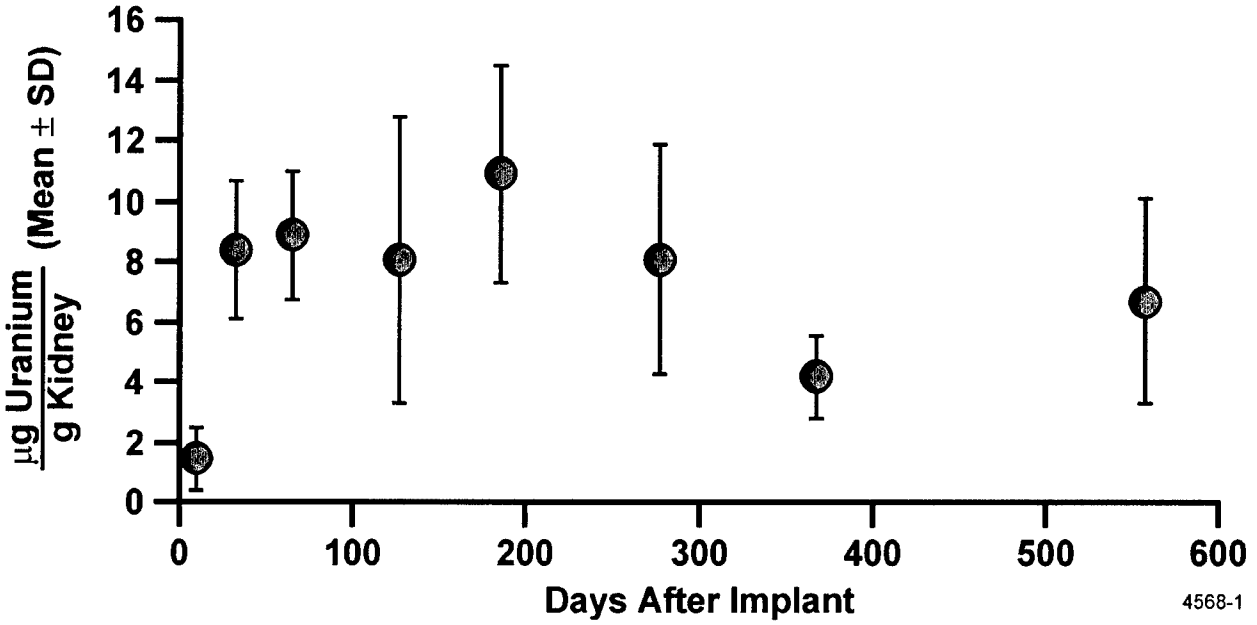

Figure 28. Renal concentration of U in rats sacrificed at intervals from 10 to 550 days after implants of 2.5 × 2.5 mm DU fragments.

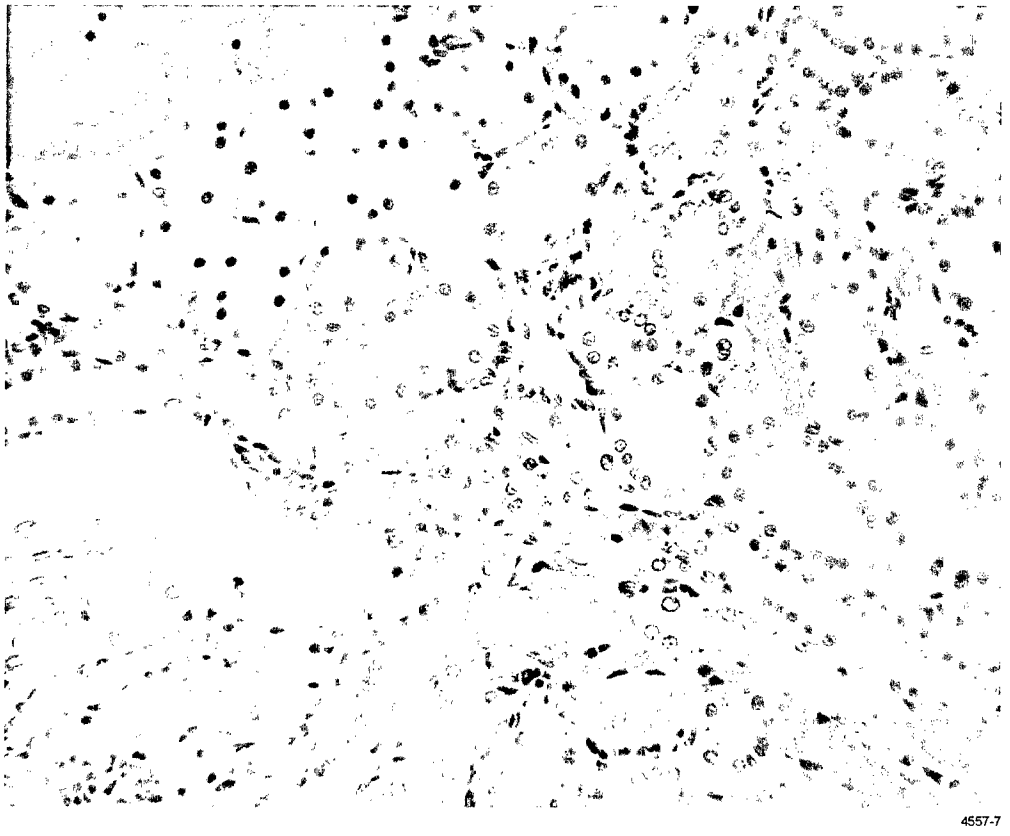

Figure 29. Renal tubular regeneration 33 days after implantation with 2.5 × 2.5 mm DU(Ti) fragments (rat T048-6042).

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 19 Feb 02

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

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Encl

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Deputy Chief of Staff for
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